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INKAPSULACIJA BIOAKTIVNIH JEDINJENJA SPOREDNIH PROIZVODA PRERADE VOĆA

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Sažetak: Svest o očuvanju životne sredine dovela je do novih trendova u prehrambenoj industriji, koji se između ostalog ogledaju i u intenzivnom izučavanju potencijala iskorišćenja otpada nastalog pri proizvodnji hrane. Pored toga, težnja ka zdravom načinu života doprinela je razvoju funkcionalnih prehrambenih proizvoda. Tokom prerade voća mnogi delovi ploda, kao što su pokožica, semenke i koštice, zaostaju, što predstavlja problem sa ekološkog i ekonomskog aspekta. S druge strane, ovi ostaci predstavljaju potencijalne izvore bioaktivnih jedinjenja. U tom pogledu, nastali sporedni proizvodi prerade voća se intenzivno izučavaju kao sirovine za ekstrakciju fenolnih jedinjenja, prirodnih pigmenata, dijetetskih vlakana, proteinskih izolata i ulja, kao i za proizvodnju suplemenata sa potencijalnim zdravstvenim benefitima. Ipak, kritičan faktor uspešne implementacije ekstrakata bogatih bioaktivnim jedinjenjima u prehrambene proizvode jeste njihova podložnost degradaciji. Prema tome, kao glavni izazov nameće se postizanje stabilnosti bioaktivnih jedinjenja tokom prerade i skladištenja, odnosno očuvanje njihove bioaktivnosti i biodostupnosti. Odgovor na postavljeni izazov očuvanja bioaktivnosti sastojaka hrane može da ponudi inkapsulacija. Naime, inkapsulacija se bazira na formiranju fizičke barijere između bioaktivnih jedinjenja i različitih neželjenih faktora sredine, kao što su visoka temperatura, svetlost, itd. U ovom radu ukazano je na potencijal sporednih proizvoda prerade jabuka, grožđa, šljiva, malina i višanja kao izvora bioaktivnih jedinjenja. Takođe, prikazane su prednosti koje se postižu inkapsulacijom bioaktivnih jedinjenja ekstrahovanih iz sporednih proizvoda prerade voća u cilju razvoja novih, funkcionalnih proizvoda.

Ključne reči: sporedni proizvodi, prerada voća, inkapsulacija, bioaktivna jedinjenja, jabuka, grožđe, šljiva, malina, višnja.

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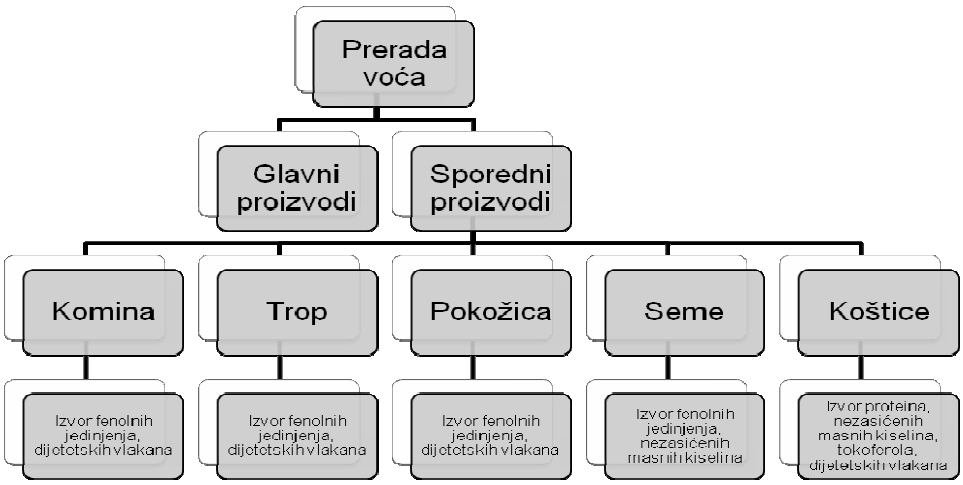
Uvod

Koncept funkcionalne hrane predstavlja rezultat intenzivnih zahteva potrošača za razvojem prehrambenih proizvoda koji će doprineti zdravom životu. U okviru ovog koncepta hrana se više ne posmatra samo sa aspekta unosa neophodnih nutrijenata i zadovoljavanja gladi, već dobija novu, specifičnu ulogu koja se ogleda u smanjenju rizika pojave bolesti i poboljšanja fizičkog i mentalnog stanja potrošača (Menrad, 2003). U tom pogledu, voće se ističe kao bogat izvor raznovrsnih sastojaka koji ispoljavaju pozitivne efekte po zdravlje (Đilas et al., 2009).

Prema Organizaciji za hranu i poljoprivredu (engl. *Food and Agriculture Organization of the United Nations*, FAO) tokom različitih faza u lancu snabdevanja hranom gotovo jedna trećina hrane se odbacuje ili gubi, što iznosi oko 1.300 milijardi kg godišnje (Gustavsson et al., 2011). Veliki udeo čine sporedni proizvodi nastali tokom procesa prerade, što predstavlja problem sa ekološkog i ekonomskog aspekta. Ipak, intenzivna izučavanja ovih rezidua ukazuju na njihov potencijal kao pristupačnih sirovina za proizvodnju prirodnih prehrambenih aditiva, odnosno za razvoj proizvoda sa dodatom vrednošću (slika 1) (Galanakis, 2012). U tom pogledu, sporedni proizvodi prerade voća privlače veliku pažnju kao sirovine za proizvodnju dijetetskih suplemenata sa visokom nutritivnom i biološkom vrednošću (Schieber et al., 2001; Đilas et al., 2009; Mirabella et al., 2014).



Slika 1. Sporedni proizvodi prerade voća kao izvori bioaktivnih jedinjenja.
Figure 1. Bioactive compounds derived from fruit processing by-products.

Kritičan faktor dodavanja ekstrakata bogatih bioaktivnim jedinjenjima u prehrambene proizvode predstavlja njihova nestabilnost (Đorđević et al., 2015). Naime, bioaktivna jedinjenja su podložna promenama i inaktivaciji usled dejstva različitih faktora tokom procesa prerade, skladištenja (temperatura, kiseonik, svetlost) ili u gastrointestinalnom traktu (pH vrednost, enzimi). Dakle, proizvođači prehrambenih proizvoda treba da obezbede zaštitni mehanizam koji će očuvati funkcionalnost ovih jedinjenja do konzumiranja i potom omogućiti kontrolisano otpuštanje na ciljnom mestu u organizmu (Nedović et al., 2011). Navedeni zahtevi mogu biti postignuti primenom inkapsulacije. Naime, proces inkapsulacije predstavlja pristup očuvanja širokog spektra bioaktivnih jedinjenja formiranjem fizičke barijere koja ih štiti od nepoželjnih faktora iz spoljašnje sredine, doprinoseći poboljšanju njihove stabilnosti i biodostupnosti *in vivo* i *in vitro* (Nedović et al., 2011; Đorđević et al., 2015).

U ovom radu ukazano je na prisustvo bioaktivnih jedinjenja u sporednim proizvodima prerade voća koje se u Srbiji proizvodi u značajnim količinama (tabela 1). Takođe, opisani su potencijali primene inkapsulacije u cilju zaštite bioaktivnih jedinjenja uz pregled do sada razvijenih funkcionalnih proizvoda obogaćenih inkapsulatima na bazi ovih komponenti.

Tabela 1. Količine proizvedenog voća u svetu i Srbiji 2016. godine (FAOSTAT, 2018).
Table 1. Fruit production quantity worldwide and in Serbia in 2016 (FAOSTAT, 2018).

Voće *Fruit*	Proizvedeno u svetu (milion kg) *World production (million kg)*	Proizvedeno u Srbiji (milion kg) *Production in Serbia (million kg)*
Jabuka	89.329,18	328,37
Grožđe	77.438,93	145,83
Šljiva	12.050,80	463,12
Malina	795,25	61,88
Višnja	1.378,22	80,60

Bioaktivna jedinjenja sporednih proizvoda prerade voća

Jabuka (*Malus domestica* L.)

Tokom procesa prerade jabuka zaostaju pokožica, semenke i jezgro, odnosno usitnjen mesnati deo. Oko 20% nastalih sporednih proizvoda se koristi kao stočna hrana, dok ostalih 80% predstavlja gubitak biomase i problem sa aspekta upravljanja otpadom. Napredak u tehnologiji otvorio je mogućnosti njihovog

iskorišćenja za proizvodnju organskih kiselina (limunska, mlečna), enzima (celulaza, hemicelulaza, lignolitički enzimi, amilaze, pektinaze), i prirodnih antioksidanasa (fenolna jedinjenja). Trop jabuke može se koristiti i kao izvor rastvorljivih i nerastvorljivih dijetetskih vlakana. Dominantni konstituenti vlakana su pektin, celuloza, hemiceluloza, lignini i gume (Dhillon et al., 2013).

Oko 75% mase ploda jabuke se iskoristi za dobijanje soka. Ostalih 25% mase ploda zaostaje u obliku tropa koji ima veliki potencijal kao sirovina za proizvodnju raznih nutrijenata kao što su ugljeni hidrati, proteini, vlakna, vitamini i minerali (Shalini i Gupta, 2010). Pored toga, trop jabuke sadrži i velike količine ukupnih fenolnih jedinjenja, ukupnih flavonoida i ukupnih flavan-3-ola koji direktno doprinose snažnoj antioksidativnoj aktivnosti *in vitro* (Ćetković et al., 2008). Analiza fenolnih jedinjenja ukazala je na prisustvo kafeinske i hlorogenske kiseline, (+)-katehina i (-)-katehina, epikatehina, epikatehin dimera (procijanidin B2), trimera, tetramera i oligomera, rutina, kvercetin glikozida, florizina, 3-hidroksiflorizina (Lu i Foo, 2000; Ćetković et al., 2008). Rezultati testova antioksidativne aktivnosti pokazali su da fenolna jedinjenja tropa jabuke imaju 2–3 puta veću sposobnost neutralisanja DPPH radikala, odnosno 10–30 puta veću sposobnost neutralisanjasuperoksid anjon radikala u poređenju sa vitaminima C i E. U skupini fenolnih jedinjenja procijanidini i kvercetin glikozidi su ispoljili najsnažniju antioksidativnu aktivnost (Lu i Foo, 2000).

Pokožica, jezgro, semenke i trop zaostali tokom prerade jabuka su poređeni u pogledu sadržaja ukupnih fenolnih jedinjenja. Pokožica sadrži najveću količinu i to preko sedam puta veću u odnosu na trop, do pet puta veću u odnosu na semenke, odnosno deset puta veću u odnosu na jezgro. Dominantni flavonoli pokožice su kvercetin-3-*O*-galaktozid praćen kvercetin-3-*O*-ramnozidom i kvercetin-3-*O*-glukozidom. U ispitivanim sporednim proizvodima jabuke uočeno je prisustvo florizina, koji u slučaju semenki iznosi 85%, odnosno u slučaju jezgra 60% ukupnih fenolnih jedinjenja. Ustanovljeno je da pokožica sadrži i najveću količinu epikatehina kao najzastupljenijeg flavan-3-ola. Dominantna fenolna kiselina je hlorogenska, dok je cijanidin-3-*O*-galaktozid najzastupljeniji predstavnik antocijana (Vasantha Rupasinghe i Kean, 2008).

Primera radi, u Kanadi se procenjuje da se prilikom proizvodnje pite od jabuka godišnje odbacuje oko 2–3.000.000 kg pokožice, iz koje se može izolovati čak 500–1.000 kg fenolnih jedinjenja (Vasantha Rupasinghe i Kean, 2008). Istraživanja su pokazala da su ekstrakti pokožice jabuke efikasni inhibitori oksidacije polinezasićenih masnih kiselina (Huber i Vasantha Rupasinghe, 2009).

Dakle, sporedni proizvodi prerade jabuka se mogu koristiti kao izvor nutrijenata i prirodnih antioksidanata. Njihov dodatak prehrambenim proizvodima doprineo bi sprečavanju neželjenih reakcija oksidacije indukovanih dejstvom slobodnih radikala, što bi imalo pozitivan uticaj na kvalitet i održivost samog proizvoda, kao i na zdravlje potrošača.

Grožđe (*Vitis vinifera* L.)

Oko 80% svetske proizvodnje grožđa se prerađuje u vino. Procenjuje se da oko 20% mase grožđa zaostaje kao komina (Schieber et al., 2001). Sporedni proizvodi proizvodnje vina uglavnom se koriste u malim količinama i to kao kondicioneri zemljišta uprkos tome što se mogu koristiti kao supstrat za proizvodnju etanola, tartarata, malata, limunske kiseline, ulja semenki i dijetetskih vlakana (Kammerer et al., 2014).

Komina je prepoznata kao vredan izvor fenolnih jedinjenja koja se u maloj meri ekstrahuju iz pokožice i semenki tokom vinifikacije. Profil i sadržaj fenolnih jedinjenja varira u zavisnosti od sorte grožđa, uslova i načina uzgajanja vinove loze, zrelosti grožđa, kao i tehnoloških parametara primenjenih tokom vinifikacije (Kammerer et al., 2014). Kammerer et al. (2004) su u svojoj studiji izvršili identifikaciju i kvantifikaciju širokog spektra fenolnih jedinjenja u pokožici i semenkama grožđa izolovanim iz sporednih proizvoda vinarija. Ukazano je na prisustvo 13 antocijana (dominantan je malvidin-3-*O*-glukozid, praćen peonidin-3-*O*-glukozidom), 11 fenolnih kiselina (najdominantnija je kaftarna kiselina), 13 flavonola, kao i 2 stilbena. Pored toga, pokazano je da su antocijani, fenolne kiseline, flavanol i flavonol glikozidi koncentrisani uglavnom u pokožici, a flavanoli u semenkama. Antocijani su u velikim količinama prisutni u pokožici crnih sorti grožđa, kao što su recimo prokupac, najrasprostranjenija autohtona sorta Srbije, i *cabernet sauvignon,* jedna od najprisutnijih, najcenjenijih i najčešće prerađivanih crnih sorti (Kalušević et al., 2015; Kalušević et al., 2016a; Veljović, 2016). Osim nedostatka antocijana kod belog grožđa, druge značajne razlike između crnih i belih sorti nisu primećene (Kammerer et al., 2004).

Ispitivanjem sastava tropa i peteljki grožđa pokazano je da sadrže velike količine dijetetskih vlakana koja čine i do ¾ suve materije. Pored toga, trop predstavlja i izvor proteina. Visok sadržaj ukupnih fenolnih jedinjenja i odlična antioksidativna svojstva su zapažena i za trop i za peteljke, a naročito za peteljke (Llobera i Cañellas, 2007).

Pokožica grožđa predstavlja dobar izvor mikro i makroelemenata. U pojedinim studijama pokožica grožđa analizirana je u pogledu sadržaja 30 elemenata. Nivoi toksičnih i potencijalno toksičnih elemenata bili su veoma niski ili ispod praga detekcije ukazujući na bezbednost primene pokožice grožđa kao sastojka dijetetskih suplemenata (De Nisco et al., 2013; Salević et al., 2017).

Proizvodnja ulja iz semenki grožđa predstavlja efikasno upravljanje sporednim proizvodima i alternativu tradicionalno korišćenim jestivim biljnim uljima. Prisustvo nezasićenih masnih kiselina (posebno linoleinske) i prirodnih antioksidanasa (fenolnih jedinjenja i tokoferola) čini ulje semenki grožđa izuzetno vrednim proizvodom. Pokazano je da fenolna jedinjenja poboljšavaju oksidativnu stabilnost i antioksidativni kapacitet ulja što doprinosi očuvanju kvaliteta i

nutritivne vrednosti štiteći od pojave neprijatnog ukusa i degradacije lipida (Malićanin et al., 2014).

Jedni od potencijalnih korisnika sporednih proizvoda prerade grožđa mogli bi biti pekarska i konditorska industrija. Primera radi, Pasqualone et al. (2014) su u svojoj studiji razvili funkcionalne biskvite sa dodatkom ekstrakta komine grožđa. Analiza proizvoda pokazala je da obogaćeni biskviti ispoljavaju snažniju antioksidativnu aktivnost i sadrže veće količine ukupnih fenolnih jedinjenja, flavonoida, antocijana i proantocijanidina u odnosu na biskvite bez dodatka ekstrakta. Na senzornom nivou zapaženi su intenzivnija boja, voćni miris i kiseo ukus obogaćenog biskvita, ali bez uticaja na prihvatljivost proizvoda.

Navedena istraživanja ukazuju da se sporedni proizvodi prerade grožđa mogu koristiti kao jeftine sirovine za ekstrakciju fenolnih jedinjenja u cilju proizvodnje prirodnih prehrambenih pigmenata i/ili antioksidanasa ili pak za proizvodnju visokokvalitetnih ulja.

Šljiva (*Prunus domestica* L.)

Pokožica šljive predstavlja još jedan sporedni proizvod prerade voća bogat fenolnim jedinjenjima. Boja pokožice šljive varira od zelene, preko žute do različitih crvenih i plavih nijansi u zavisnosti od biosinteze i akumulacije fenolnih pigmenata antocijana. Pored ovih pigmenata, pokožica sadrži i širok spektar neobojenih fenolnih jedinjenja. Naime, ispitivanjem varijabilnosti fenolnog profila pokožice različitih varijeteta šljive izvršena je identifikacija i kvantifikacija 49 fenolnih jedinjenja. Antocijani su dominantna grupa fenolnih jedinjenja (odnos ukupni antocijani/ukupna fenolna jedinjenja je veći od 50% za većinu varijeteta). Najzastupljeniji antocijani su glikozidi cijanidina i peonidina. U značajnim količinama prisutni su flavonoli (najzastupljeniji je rutin) i hidroksicinamične kiseline (neohlorogenska kiselina je dominantna). Pored navedenih klasa, pokožica šljive sadrži i flavone i acilovane flavonoide (Treutter et al., 2012).

Pored pokožice, istraživanja su pokazala da i trop šljive predstavlja sirovinu od interesa za ekstrakciju fenolnih jedinjenja. Sójka et al. (2015) su ukazali na veoma visok sadržaj ukupnih fenolnih jedinjenja u ekstraktima tropa šljive (do 50 g/100 g) i veliki antioksidativni kapacitet. Pripremljeni ekstrakti sadrže i velike količine antocijana (cijanidin i peonidin rutinozidi su glavne komponente), hidroksicinamičnih kiselina, flavanola i flavonol glikozida. Rezultati navedene studije ukazali su i na baktericidne efekte prema bakteriji *Lysteria monocytogenes*. Milala et al. (2013) su u svojoj studiji ukazali na dodatnu vrednost tropa šljive kao izvora dijetetskih vlakana (38–49% suve mase). Trop šljive sadrži ugljene hidrate (16–22% suve mase) pri čemu glukoza čini do 50%, fruktoza 20–40% i sorbitol do 10% suve mase ukupnih ugljenih hidrata (Milala et al., 2013).

Tokom prerade šljive zaostaju koštice, čije dalje iskorišćenje takođe predstavlja predmet istraživanja. González-García et al. (2014) su iz koštica šljive izolovali proteinsku frakciju sa sadržajem proteina oko 40%, a daljom analizom identifikovano je 13 bioaktivnih peptida sa antioksidativnom i antihipertenzivnom aktivnošću. Istraživanja pokazuju da se koštica šljive može koristiti i za proizvodnju ulja. Karakterizacijom ulja koštica šljive identifikovane su njegove glavne komponente: alkan hentrikontan, estar etil heksadekanoat i linoleinska kiselina. Pored toga, pokazano je da ovo ulje ima snažnu antioksidativnu aktivnost, umerenu antibakterijsku aktivnost prema *Salmonella typhi* i umerenu antifungalnu aktivnost prema *Microsporum canis* (Mahmood et al., 2009).

Dakle, sporedni proizvodi prerade šljiva predstavljaju sirovine koje se mogu koristiti za proizvodnju preparata sa visokim sadržajem fenolnih jedinjenja i dijetetskih vlakana, proteinskih izolata, prirodnih pigmenata ili pak za proizvodnju ulja.

Malina (*Rubus idaeus* L.)

Tokom prerade malina u sok nastaje velika količina otpada koji se sastoji od pulpe i semenki. Nastali sporedni proizvodi predstavljaju obećavajući izvor jedinjenja sa pozitivnim nutritivnim i tehnološkim svojstvima. Istraživanja su pokazala da ekstrakti dobijeni iz sporednih proizvoda prerade malina u sok sadrže značajne količine ukupnih fenolnih jedinjenja, flavonoida i antocijana, kao i da ispoljavaju dobru sposobnost neutralizacije DPPH radikala (Četojević-Simin et al., 2015; Kalušević et al., 2016b). Dodatnoj vrednosti ovih ekstrakata kao potencijalnih sastojaka funkcionalne hrane doprinose antiproliferativna i proapoptotska aktivnost, kao i sposobnost inhibicije bakterijskog rasta u niskim koncentracijama (Četojević-Simin et al., 2015).

Trop maline je veoma bogat izvor dijetetskih vlakana, koja čine 77,5% suve mase. Iz tog razloga, ispitivan je potencijal osušenog tropa maline sa izdrobljenim i neizdrobljenim semenkama za zamenu brašna na nivou 25 i 50% pri proizvodnji keksa kao jedan od načina za povećanje sadržaja dijetetskih vlakana u ishrani. Navedena zamena imala je pozitivan uticaj na sadržaj vlakana u keksu, a najveća količina vlakana određena je u uzorku sa dodatkom 50% tropa sa neizdrobljenim semenkama. Sa povećanjem sadržaja tropa u formulaciji za ove proizvode zapaženi su voćni miris i voćno-kiseli ukus uz smanjenje slatkog ukusa. Generalno, keks sa neizdrobljenim semenkama je okarakterisan kao najprihvatljiviji ne samo u pogledu sadržaja ukupnih dijetetskih vlakana, već i na senzornom nivou (Górecka et al., 2010).

Još jedan primer iskorišćenja sporednih proizvoda voća u konditorskoj industriji ogleda se u razvoju inovativnog bezglutenskog keksa. Naime, Šarić et al. (2016) su u svojoj studiji ispitali potencijal zamene dela brašna u formulaciji

bezglutenskog keksa proizvodom dobijenim od osušenih i usitnjenih sporednih proizvoda maline i borovnice. Na ovaj način proizveden je keks sa dodatom vrednošću koji se pored većeg sadržaja fenolnih jedinjenja, dijetetskih vlakana i minerala, ističe i smanjenim sadržajem masti i poboljšanim sastavom masnih kiselina u odnosu na komercijalno dostupan bezglutenski keks. Keks u kome je 28,2% brašna zamenjeno tropom borovnice, a 1,8% tropom maline ocenjen je kao najprihvatljiviji sa aspekta nutritivnog kvaliteta i senzornih svojstava.

Semenke malina mogu se koristiti za proizvodnju ulja sa potencijalnim nutraceutičkim efektima. U studiji publikovanoj od strane Oomah et al. (2000) postignut je prinos ulja 10,7%. Analiza fizičkih i hemijskih svojstava ukazala je na visok kvalitet ekstrahovanog ulja. Naime, dobijeno je ulje žute boje, niske viskoznosti, otporno prema oksidaciji i stabilno tokom skladištenja. Sadrži značajne količine karotenoida i bogato je α-, γ- i δ-tokoferolom. Lipidna frakcija sadrži 93,7% neutralnih lipida, 3,5% fosfolipida i 2,7% slobodnih masnih kiselina. Linolna, α-linoleinska i oleinska kiselina zajedno čine 96% ukupnih masnih kiselina ulja semenki maline.

Lišće maline ne predstavlja sporedni proizvod nastao tokom procesa proizvodnje soka, već zaostaje tokom berbe ili se u maloj meri sakuplja i primenjuje za pripremu čaja. Ipak, lišće maline se može koristiti za proizvodnju vodenih i alkoholnih ekstrakata bogatih ukupnim fenolnim jedinjenjima, flavonoidima i flavan-3-olima. Rezultati različitih testova određivanja antioksidativne aktivnosti ukazali su da ovi ekstrakti ispoljavaju snažnu sposobnost neutralisanja slobodnih radikala (Venskutonis et al., 2007; Salević et al., 2016).

Liofilizovani i koncentrovani oblici ekstrakata lišća maline dodavani su različitim vrstama čokolade kao aktivni sastojci. Povećanje sadržaja ukupnih fenolnih jedinjenja, flavan-3-ola i proantocijanidina, kao i veći antioksidativni kapacitet obogaćenih čokolada postignuti su dodavanjem 3% koncentrovanog ekstrakta. Međutim, senzorna analiza pokazala je da je dodavanje ekstrakta u liofilizovanom obliku poželjnije sa aspekta ukusa, vizuelnih i teksturnih svojstava (Belščak-Cvitanović et al., 2012).

Rezultati dosadašnjih istraživanja pokazuju da se sporedni proizvodi prerade malina mogu koristiti za proizvodnju prirodnih pigmenata, ulja i dijetetskih suplemenata bogatih prirodnim antioksidansima i dijetetskim vlaknima, odnosno za razvoj širokog spektra inovativnih funkcionalnih proizvoda.

Višnja (*Prunus cerasus* L.)

Sporedni proizvodi koji nastaju tokom prerade višanja čine 15□28% mase sirovine zavisno od tehnološkog procesa proizvodnje. Uprkos veoma dobrom potencijalu ovih sporednih proizvoda za dalju preradu, odnosno proizvodnju ekstrakata bogatih fenolnim jedinjenjima i dijetetskim vlaknima, oni se koriste

uglavnom kao ogrev ili stočna hrana. Ekstrakti tropa višnje su okarakterisani kao bogati izvori fenolnih jedinjenja uključujući antocijane, hidroksicinamične kiseline i flavanole (Kołodziejczyk et al., 2013). U pogledu individualnih fenolnih jedinjenja najzastupljeniji su cijanidin-3-glikozilrutinozid, neohlorogenska kiselina i katehin (Yılmaz et al., 2015). Ekstrakti tropa višnje ispoljavaju antioksidativnu i antimikrobnu aktivnost. Kao antimikrobni agensi ekstrakti tropa višnje mogu preventivno delovati na bolesti uzrokovane patogenim bakterijskim vrstama poreklom iz hrane: *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhymurium* i *Bacillus cereus* (Kołodziejczyk et al., 2013; Demirdöven et al., 2015).

Tokom prerade višanja odbacuju se koštice. Ispitivanjem hemijskog sastava ekstrakata koštica višnje ukazano je na prisustvo ulja (17%), proteina (29,3%) i dijetetskih vlakana (30,3%). Analizom hemijskog sastava pokazano je da ulje koštice višnje predstavlja bogat izvor bioaktivnih komponenti kao što su polinezasićene masne kiseline, tokoferoli, β-karoten i fenolna jedinjenja. Dominantne masne kiseline su oleinska, linoleinska, palmitinska, linolna i stearinska (Yılmaz i Gökmen, 2013).

Górnaś et al. (2016) su koristili sporedne proizvode prerade voća, tropove višnje, maline, jagode i crne ribizle, kao izvore raznovrsnih fenolnih jedinjenja za proizvodnju mafina sa dodatom vrednošću. Rezultati navedene studije su ukazali na neophodnost optimizacije procesa proizvodnje u cilju očuvanja stabilnosti fenolnih jedinjenja. Naime, termički proces pripreme mafina doveo je do degradacije fenolnih jedinjenja, pri čemu su najveću nestabilnost ispoljili antocijani, dok su flavonol glikozidi okarakterisani kao najstabilniji. U tom pogledu ustanovljeno je da je za očuvanje stabilnosti ovih jedinjenja najbolje primeniti više temperature tokom kraćeg vremena pečenja. Obogaćivanje mafina raznim vrstama tropa voća bogatih fenolnim jedinjenjima nije uticalo na njihovu senzornu prihvatljivost.

Na osnovu navedenog može se zaključiti da su sporedni proizvodi prerade višnje još jedna pristupačna sirovina koja se može koristiti kao pogodna alternativa sintetičkih aditiva čime se poboljšava funkcionalnost prehrambenih proizvoda.

Ostalo voće

Efikasno upravljanje sporednim proizvodima prerade raznovrsnog voća, pored navedenih vrsta, privlači veliku pažnju kroz različite studije ispitivanja njihovog hemijskog sastava i formulacije inovativnih prehrambenih proizvoda sa dodatom vrednošću (Kalušević et al., 2016c). Na primer, tokom prerade citrusa u sok zaostaju kora, pulpa i semenke, koji su bogati fenolnim jedinjenjima iz klase flavonoida (Lauro et al., 2015). Tokom proizvodnje soka od nara zaostaje kora bogata fenolnim jedinjenjima od kojih su najzastupljeniji punikalagin i elaginska

kiselina (Çam et al., 2014). Drugi sporedni proizvod prerade nara čine semenke koje se mogu koristiti za ekstrakciju ulja (Goula i Adamopoulos, 2012). Sporedni proizvodi nastali tokom prerade raznih vrsta voća, kao što su borovnica (Flores et al., 2014), crna ribizla (Bakowska-Barczak i Kolodziejczyk, 2011) i crni dud (Gültekin-Özgüven et al., 2016) okarakterisani su visokim sadržajem fenolnih jedinjenja, naročito antocijana.

Inkapsulacija bioaktivnih jedinjenja

Bioaktivna jedinjenja su podložna degradaciji ili inaktivaciji usled dejstva različitih faktora tokom procesa prerade i skladištenja (svetlost, temperatura, kiseonik), kao i u gastrointestinalnom traktu (pH vrednost, enzimi). U tom pogledu, za razvoj funkcionalnih prehrambenih proizvoda obogaćenih bioaktivnim jedinjenjima inkapsulacija predstavlja efikasan pristup postizanja stabilnosti i kontrolisanog otpuštanja ovih jedinjenja, odnosno očuvanja njihove funkcionalnosti (Champagne i Fustier, 2007; de Vos et al., 2010; Nedović et al., 2011).

Inkapsulacija se može definisati kao proces „hvatanja" aktivne komponente u nosač ili formiranja fizičke barijere između okolne sredine i aktivne komponente u cilju njene zaštite (Nedović et al., 2011; Nedović et al., 2013). Sistem aktivna komponenta/nosač naziva se inkapsulat. U zavisnosti od odabira nosača i tehnike inkapsulacije mogu se dobiti čestice različitog oblika i veličine od nekoliko nanometara do nekoliko milimetara (Fang i Bhandari, 2010; Lević et al., 2014a).

Primenom inkapsulacije mogu se postići brojne prednosti kao što su:

- stabilizacija aktivne komponente i očuvanje funkcionalnosti tokom prerade, skladištenja i konzumiranja;
- smanjenje stope degradacije aktivne komponente uzrokovane dejstvom različitih nepovoljnih spoljašnjih faktora ili reakcijom sa drugim komponentama proizvoda;
- postizanje kontrolisanog otpuštanja aktivne komponente pravovremeno na ciljnom mestu;
- maskiranje neželjenog ukusa (npr. astrigencija fenolnih jedinjenja);
- omogućavanje lakšeg rukovanja aktivnom komponentom (npr. prevođenje tečnih biljnih ekstrakata u čvrste čestice);
- postizanje adekvatne koncentracije i uniformne raspodele aktivne komponente, i
- stvaranje vizuelnih i teksturnih efekata (Fang i Bhandari, 2010; Zuidam i Shimoni, 2010; Nedović et al., 2011; Nedović et al., 2013).

Zbog navedenih prednosti inkapsulacija privlači sve više pažnje kao značajna faza pri formulaciji zdravijih i ukusnijih prehrambenih proizvoda bez obzira na to

što se njenim uvođenjem povećavaju troškovi i složenost procesa proizvodnje (Zuidam i Shimoni, 2010).

Nosači za inkapsulaciju

Izbor nosača za inkapsulaciju aktivnih komponenti i razvoj funkcionalnih prehrambenih proizvoda se svodi na materijale sa statusom GRAS (engl. *Generally Recognized As Safe*), koji se nalaze na listi dozvoljenih aditiva za primenu u hrani. Odabrani materijal mora biti biodegradabilan i sposoban da formira barijeru između unutrašnje faze i njenog okruženja obezbeđujući maksimalnu zaštitu inkapsulisane komponente i zadržavajući je u strukturi matriksa tokom procesa prerade ili skladištenja. Najvažniji kriterijumi prilikom izbora su svojstva aktivne komponente, tehnika inkapsulacije, cilj inkapsulacije, funkcionalnost inkapsulata u proizvodu, fizičke i hemijske karakteristike nosača, način otpuštanja inkapsulisane komponente, zahtevi u pogledu stabilnosti i cena (Nedović et al., 2011; Nedović et al., 2013).

Na raspolaganju je veliki broj različitih materijala:

- polisaharidi:
  - biljnog porekla: skrob i derivati skroba (npr. maltodekstrini, ciklodekstrini), celuloza i derivati celuloze (npr. etil celuloza), ekstrudati i ekstrakti (npr. arapska guma, pektini, galaktomanani),
  - morskog porekla: karagenan, alginat,
  - mikrobnog/animalnog porekla: ksantan, gelan, dekstran, hitozan;
- proteini:
  - biljnog porekla: gluten, proteinski izolati (graška, soje),
  - animalnog porekla: kazein, proteini surutke, želatin;
- lipidi:
  - biljnog porekla: masne kiseline i alkoholi, gliceridi, voskovi (npr. karnauba), fosfolipidi,
  - mikrobnog/animalnog porekla: masne kiseline i alkoholi, gliceridi, voskovi (npr. pčelinji), fosfolipidi;
- ostali: polivinilpirolidon, parafin, neorganski materijali (npr. tripolifosfat, oksidi silicijuma) (Wandrey et al., 2010).

U cilju dobijanja inkapsulata sa što boljim karakteristikama moguće je kombinovanje različitih prirodnih materijala (npr. sistemi na bazi alginatnih čestica i lipozoma, Balanč et al., 2016) ili oblaganje, npr.alginatnih i pektinskih hidrogel čestica hitozanom (Belščak-Cvitanović et al., 2017). Takođe, moguće je kombinovati prirodne i sintetičke materijale kao nosače aktivnih komponenti (npr. alginat i polivinil alkohol) (Lević et al., 2011).

Tehnike inkapsulacije

Razvijen je veliki broj tehnika koje se mogu primeniti za inkapsulaciju aktivnih komponenti. Najvažniji kriterijumi kod izbora odgovarajuće tehnike inkapsulacije su: cilj inkapsulacije, fizička, hemijska i biološka svojstva aktivne komponente, fizička i hemijska svojstva nosača, vrsta proizvoda u koji će se inkapsulat dodavati, uslovi skladištenja inkapsulata i proizvoda obogaćenih inkapsulatima, mehanizam otpuštanja i cena (Desai i Jin Park, 2005; Zuidam i Shimoni, 2010).

Sprej sušenje je jedna od najstarijih i najčešće primenjivanih tehnika inkapsulacije u prehrambenoj industriji. Ova tehnika je veoma pogodna za inkapsulaciju aktivnih komponenti zbog fleksibilnosti, kontinualnosti, visoke stope produktivnosti i ekonomičnosti pri čemu se dobijaju suvi i stabilni prahovi dobrog kvaliteta (Desai i Jin Park, 2005; Fang i Bhandari, 2010; Nedović et al., 2013). Može se koristiti za inkapsulaciju bilo hidrofilnih ili hidrofobnih aktivnih komponenti, dok je izbor nosača ograničen na hidrofilne materijale (Nedović et al., 2013). Sprej sušenje se zasniva na atomizaciji disperzije ili emulzije aktivne komponente i nosača u struji zagrejanog vazduha. Pri kontaktu sa vrelim vazduhom rastvarač brzo isparava i nastaju čestice koje se odvajaju na nižoj izlaznoj temperaturi (Augustin i Hemar, 2009). U zavisnosti od primenjenog nosača mogu se dobiti čestice/prahovi prečnika od nekoliko do 300 μm (Belščak-Cvitanović et al., 2015; Đorđević et al., 2015).

Sprej rashlađivanje/hlađenje su tehnike inkapsulacije aktivnih komponenti u lipidne matrikse (Nedović et al., 2011). Ove tehnike su jeftine, brze, kontinualne i reproduktivne, a jedina razlika između sprej hlađenja i rashlađivanja je u tački topljenja lipida. Naime, u slučaju sprej hlađenja kao nosači se koriste rastopi frakcionisanih i hidrogenizovanih biljnih ulja (tačka topljenja 32–42°C), dok se biljna ulja i drugi materijali (tačka topljenja 45–122°C) koriste za sprej rashlađivanje (Đorđević et al., 2015). Princip ovih tehnika je suprotan od principa opisanog kod sprej sušenja. Naime, rastop lipida i aktivne komponente se atomizira u struji ambijentalnog ili hladnog vazduha formirajući čestice (Nedović et al., 2013). U zavisnosti od tipa atomizera i procesnih parametara mogu se dobiti čestice veličine od 50 do čak 2.000 μm (Đorđević et al., 2015).

Oblaganje u fluidizovanom sloju je tehnika oblaganja čvrstih čestica nosačem, a može se primeniti i za formiranje sekundarnog sloja inkapsulata sa veoma osetljivim aktivnim komponentama prethodno dobijenih tehnikom sprej sušenja (Augustin i Hemar, 2009). Princip tehnike se zasniva na suspendovanju aktivne komponente u struji vazduha i atomizaciji nosača na fluidizovane čestice pri čemu nastaju kapsule (de Vos et al., 2010). Na raspolaganju je veći broj nosača u odnosu na tehniku sprej sušenja (Nedović et al., 2013).

Liofilizacija predstavlja alternativu tehnike sprej sušenja i primarno je namenjena za inkapsulaciju aktivnih komponenti koje su veoma osetljive na dejstvo povišenih temperatura (Nedović et al., 2013). Princip inkapsulacije liofilizacijom se zasniva na smrzavanju smeše aktivne komponente i nosača na veoma niskim temperaturama, a potom se snižava pritisak i dovodi toplota, pri čemu dolazi do sušenja i sublimacije rastvarača (Zuidam i Shimoni, 2010). Uprkos jednostavnosti, primena ove tehnike ograničena je na veoma vredne i osetljive komponente, jer u poređenju sa sprej sušenjem proces liofilizacije duže traje, veći je utrošak energije, a samim tim i cena procesa, dok dobijene čestice imaju porozniju strukturu, usled čega je zaštita inkapsulisane komponente manja (Augustin i Hemar, 2009; Nedović et al., 2013).

Ekstruzione tehnike privlače veliku pažnju za proizvodnju polimernih čestica (Lević et al., 2014a). Princip ovih tehnika zasniva se na ekstruziji smeše aktivne komponente i nosača kroz mlaznicu ili male otvore, pri čemu nastaju kapljice koje očvršćavaju i formiraju kapsule fizičkim (npr. zagrevanje) ili hemijskim procesom (npr. geliranje, tj. hemijsko umrežavanje) (Đorđević et al., 2015). U zavisnosti od mehanizma formiranja kapljica razlikuju se sledeće ekstruzione tehnike: tehnika ukapavanja, vibraciona tehnika, tehnika ukapavanja uz dejstvo sekundarnog toka vazduha, presecanje mlaza pomoću rotirajućeg diska i elektrostatička ekstruzija (Nedović, 1999). Najjednostavnija je tehnika ukapavanja pod dejstvom gravitacije (Lević et al., 2014a). Elektrostatička ekstruzija, zasnovana na primeni elektrostatičkog polja, je posebno pogodna tehnika za proizvodnju malih (ispod 50 μm), uniformnih čestica (Đorđević et al., 2015). Još jedna tehnika zasnovana na primeni elektrostatičkog polja je elektrospining, koja se izdvaja kao veoma efikasna tehnika za proizvodnju filmova koji se sastoje od velikog broja vlakana submikronskog reda veličine (Lević, 2014b; Echegoyen et al., 2017).

Inkapsulati bazirani na emulzijama predstavljaju atraktivne sisteme inkapsulacije lipofilnih i/ili hidrofilnih aktivnih komponenti, naročito u pogledu prevazilaženja problema vezanih za slabu rastvorljivost i stabilnost (Augustin i Hemar, 2009; Đorđević et al., 2015). Princip inkapsulacije se zasniva na dispergovanju tečnosti koja sadrži aktivnu komponentu u drugoj tečnosti sa kojom je nemešljiva (de Vos et al., 2010). U zavisnosti od prostorne organizacije uljane i vodene faze razlikuju se dva osnovna tipa emulzija: voda u ulju i ulje u vodi, a postoje i dvostruke emulzije, kao što je voda u ulju u vodi (Fang i Bhandari, 2010). Ulje u vodi emulzije se mogu sušiti raspršivanjem, a dobijene suve emulzije mogu imati primenu kao inkapsulati ili instant formule za razne prehrambene proizvode (Zuidam i Shimoni, 2010).

Lipozomi su sferne lipidne čestice, veličine od 30 nm do nekoliko mikrona, koje privlače veliku pažnju kao sistemi inkapsulacije hidrofilnih, lipofilnih i amfifilnih komponenti (Nedović et al., 2013; Đorđević et al., 2015). Razvijene su različite metode pripreme lipozoma, a mehanizam njihovog formiranja se bazira na

hidrofilno-hidrofobnim reakcijama između polarnih lipida, uglavnom fosofolipida, i molekula vode (Đorđević et al., 2015). Lipozomi se izdvajaju kao veoma efikasni sistemi kontrolisanog otpuštanja aktivne komponente na ciljnom mestu, ali ograničavajući faktor za primenu u prehrambenoj industriji je visoka cena ovakvih inkapsulacionih sistema (Fang i Bhandari, 2010; Nedović et al., 2011).

Molekulska inkluzija podrazumeva primenu materijala koji se mogu smatrati praznim kapsulama u čiju šupljinu se smešta aktivna komponenta (Zuidam i Shimoni, 2010; Đorđević et al., 2015). U tu svrhu primenjuju se ciklodekstrini, ciklični oligosaharidi izgrađeni od 6, 7 ili 8 glukopiranoznih jedinica povezanih α(1-4) vezama (Augustin i Hemar, 2009). Spoljašnjost ciklodekstrina je hidrofilna, dok je unutrašnji deo hidrofoban, pa se ciklodekstrini primenjuju za inkapsulaciju manje polarnih molekula u apolarnu unutrašnju šupljinu hidrofobnim interakcijama (Fang i Bhandari, 2010). Inkapsulacijom u ciklodekstrine postižu se odlična termička i hemijska stabilnost, kao i kontrolisano otpuštanje (Gouin, 2004). Međutim, cena i mali udeo vezane aktivne komponente su još uvek ograničavajući faktori njihove šire primene (Nedović et al., 2011).

Koacervacija je tehnika pogodna za zaštitu hidrofobnih aktivnih komponenti smeštanjem u hidrofilne omotače. Naime, princip formiranja koacervata se bazira na dispergovanju aktivne komponente u rastvoru jednog ili više polimera nakon čega se variraju parametri sistema, što dovodi do formiranja tečnog filma koji obavija aktivnu komponentu (Lević et al., 2014a). U zavisnosti od broja polimera, koacervacija može biti prosta, kada je uključen samo jedan polimer, ili kompleksna, koja se češće primenjuje, a podrazumeva korišćenje dva ili više polimera (Zuidam i Shimoni, 2010). Primenom ove tehnike može se postići veoma velika efikasnost inkapsulacije (do 99%) i kontrolisano otpuštanje aktivne komponente. Međutim, ograničavajući faktori su kompleksnost procesa, cena i mali broj materijala nosača koji se mogu koristiti, a koji su u isto vreme dozvoljeni za primenu u hrani (Gouin, 2004).

Ćelije kvasca *Saccharomices cerevisiae* kao sporedni proizvodi fermentacije predstavljaju pogodne mikrokapsule za inkapsulaciju aktivnih komponenti. Inkapsulacija u ćelije kvasca predstavlja ekonomičan proces, jer je pored nosača i aktivne komponente potrebna samo voda. Pritom, ove ćelije imaju visoku nutritivnu vrednost, a mogu se proizvesti u velikim količinama. Glavni izazov predstavlja prolazak aktivne komponente kroz membranu kvaščeve ćelije bez narušavanja strukture ćelije (Đorđević et al., 2015).

Inkapsulacija korišćenjem superkritičnih fluida zapravo predstavlja poboljšanje postojećih tehnika inkapsulacije (Zuidam i Shimoni, 2010). Najčešće se koristi ugljen-dioksid zbog zastupljenosti i niske cene (Gouin, 2004). Princip se zasniva na dispergovanju aktivne komponente u nosaču rastvorenom u superkritičnom fluidu, najčešće ugljen-dioksidu nakon čega se superkritični fluid uklanja, a aktivna komponenta ostaje zarobljena u matriksu. Proces brze ekspanzije

superkritičnih rastvora podrazumeva atomizaciju kroz mlaznice pri čemu nastaju čestice nosača sa inkapsulisanom aktivnom komponentom (Augustin i Hemar, 2009). Korišćenje superkritičnog ugljen-dioksida u procesu sprej sušenja omogućava rad na nižim temperaturama, što je naročito pogodno za inkapsulaciju veoma osetljivih aktivnih komponenti (Zuidam i Shimoni, 2010).

Inkapsulacija bioaktivnih jedinjenja sporednih proizvoda prerade voća

Ispitivanje potencijala različitih nosača i tehnika inkapsulacije kao sistema zaštite i očuvanja bioaktivnih jedinjenja izolovanih iz sporednih proizvoda prerade voća u cilju razvoja dijetetskih suplemenata i funkcionalnih prehrambenih proizvoda predstavlja veoma aktuelnu oblast istraživanja.

Trop grožđa, ostatak od proizvodnje vina, može se koristiti za pripremu ekstrakata sa visokim sadržajem fenolnih jedinjenja koji se u cilju zaštite može inkapsulisati u kalcijum-alginatne čestice primenom vibracione tehnike ekstruzije. Analiza stabilnosti inkapsulisanih i neinkapsulisanih fenolnih jedinjenja pokazala je da je inkapsulacija doprinela značajnom poboljšanju stabilnosti tokom dugoročnog skladištenja pod različitim temperaturnim uslovima i dejstvom svetlosti (Aizpurua-Olaizola et al., 2016).

Trop grožđa, zaostao pri proizvodnji crvenog vina privlači veliku pažnju za proizvodnju prahova pigmenata sa funkcionalnim svojstvima. Primenom tehnike sprej sušenja i maltodekstrina kao nosača mogu se dobiti prahovi bogati antocijanima, pri čemu se postižu dobra stabilnost i očuvanje boje tokom skladištenja. Analiza pripremljenih prahova ukazala je na sledeće karakteristike: nizak sadržaj vlage, mala higroskopnost, dobra rastvorljivost, sposobnost inhibicije rasta *Staphylococcus aureus* i *Listeria monocytogenes* i visok inhibitorni kapacitet prema enzimu arginaza (de Souza et al., 2014; de Souza et al., 2015).

Pored tehnika ekstruzije i sprej sušenja, različiti sistemi na bazi nanoemulzija imaju potencijal za inkapsulaciju ekstrakta tropa grožđa. Suncokretovo i palmino ulje mogu se koristiti kao lipidne faze za pripremu nanoemulzija. U tom pogledu, nanoemulzija pripremljena korišćenjem suncokretovog ulja predstavlja efikasan sistem zaštite fenolnih jedinjenja od fizičke i hemijske degradacije tokom skladištenja. Pored toga, pokazano je da inkapsulisana fenolna jedinjenja ispoljavaju snažniju ćelijsku antioksidativnu aktivnost, kao rezultat apsorpcije ovih jedinjenja u ćelijama, u odnosu na neinkapsulisane oblike (Sessa et al., 2013). Još jedan veoma efikasan inkapsulacioni sistem na bazi emulzija predstavlja ulje/voda nanoemulzija kod koje lipidnu fazu čine suncokretovo ulje i sojin lecitin. Ekstrakt tropa grožđa u slobodnom obliku i pripremljeni inkapsulati dodati su u pastu lešnika, čime je značajno inhibirana lipidna oksidacija tokom skladištenja. Poređenjem efikasnosti slobodnih i inkapsulisanih oblika ekstrakta pokazano je da

inkapsulacija doprinosi očuvanju antioksidativne aktivnosti i efikasnijoj inhibiciji lipidne oksidacije (Spigno et al., 2013).

Pokožica crnih sorti grožđa predstavlja veoma bogat izvor antocijana. Procedura inkapsulacije kod ovakvih sirovina se obično sastoji od pripreme ekstrakta bogatog antocijanima koji se potom inkapsuliše. Tehnika sprej sušenja se posebno pokazala pogodnom za inkapsulaciju ovakvih ekstrakata. Maltodekstrin, maltodekstrin/γ-ciklodesktrin i maltodekstrin/arapska guma su pogodni nosači za ovakve procese inkapsulacije. Dalje, pripremljeni mikroinkapsulati se mogu dodati u osvežavajuća bezalkoholna pića, a stopa degradacije antocijana tokom skladištenja u najvećoj meri zavisi od izbora nosača (Burin et al., 2011).

Bogat izvor antocijana je i ekstrakt pokožice autohtone sorte Srbije prokupac, koji se dalje može inkapsulisati i dodatno zaštititi tehnikom sprej sušenja korišćenjem maltodekstrina, arapske gume i obranog mleka u prahu kao nosača. Analiza pripremljenih prahova ukazala je na visok prinos, nisku aktivnost vode i veoma visoku rastvorljivost. Inkapsulacija je doprinela termičkoj i hemijskoj stabilnosti mikroinkapsulata uz postizanje sporijih profila otpuštanja antocijana (Kalušević et al., 2017). Tehnika liofilizacije se takođe može primeniti za inkapsulaciju ekstrakata pokožice grožđa u navedene nosače. Mikroinkapsulati pripremljeni tehnikama sprej sušenja i liofilizacije dodati su u ovsene kaše i jogurte, a rezultati senzorne analize ukazali su na prihvatljivost ovakvih proizvoda (Kalušević, 2017).

Osim navedenih nosača za inkapsulaciju ekstrakta pokožice crnog grožđa tehnikama sprej sušenja i liofilizacije mogu se primeniti i drugi nosači, kao što su guar guma i polidekstroza. Primenom tehnika sprej sušenja i liofilizacije dobijaju se mikroinkapsulati različitih fizičkih karakteristika. Naime, mikroinkapsulati pripremljeni tehnikom sprej sušenja imaju manju aktivnost vode, veću rastvorljivost, ali i veću higroskopnost u odnosu na mikroinkapsulate pripremljene tehnikom liofilizacije. Pored toga, tehnikom sprej sušenja dobijaju su čestice pravilnijeg oblika i manjeg prečnika (Kuck i Noreña, 2016).

Pored pokožice, i semenke grožđa se mogu koristiti za pripremu ekstrakta bogatog fenolnim jedinjenjima. Pripremljeni ekstrakt može se primarno inkapsulisati u lipozome i potom obložiti hitozanom u cilju postizanja što stabilnijih sistema. Oblaganje lipozoma hitozanom doprinelo je formiranju čestica većeg prečnika uz veću efikasnost inkapsulacije. Pored toga, postignuto je sporije otpuštanje fenolnih jedinjenja (Gibis et al., 2016).

Tehnika sprej sušenja može se primeniti i za inkapsulaciju ekstrakta semenki grožđa u meskit guma/zein i maltodekstrin/zein kombinacije nosača. Pripremljeni inkapsulati dodati su u keks, što je doprinelo većem antioksidativnom kapacitetu i smanjenju stepena termičke degradacije fenolnih jedinjenja tokom pečenja. Senzorna analiza je ukazala na prihvatljivost keksa sa dodatkom mikroinkapsulata (Davidov-Pardo et al., 2012).

Kao što je već ranije rečeno, pored fenolnih jedinjenja, semenke grožđa se mogu koristiti i za ekstrakciju ulja koje se potom može inkapsulisati primenom tehnike elektrostatičke ekstruzije u kalcijum-alginatne čestice. Pripremljeni inkapsulati su dodati u fermentisane kobasice u cilju smanjenja udela životinjske masti i povećanja sadržaja fenolnih jedinjenja. Analiza proizvoda pokazala je da dodatak inkapsulata nije uticao na hemijski sastav, a rezultati senzorne analize ukazali su na prihvatljivost proizvoda (Stajić et al., 2014).

Jabuka je još jedan važan izvor fenolnih jedinjenja. Tako, na primer, ekstrakt pokožice jabuke inkapsulisan je sa probiotskom bakterijom *Lactobacillus acidophilus* tehnikom koekstruzije u sferne alginatne čestice. Primena inkapsulacije uz dodatak ekstrakta pokožice jabuke kao bioaktivnog sastojka doprinela je očuvanju vijabilnosti probiotske bakterije u mleku i zakišeljenoj vodi tokom skladištenja (Shinde et al., 2014).

Ekstruzija u kombinaciji sa alginatom kao nosačem se može primeniti i za inkapsulaciju ekstrakta lišća maline bogatog fenolnim jedinjenjima. Na taj način dobijene su sferne, uniformne čestice. Dodatno oblaganje kalcijum-alginatnih čestica hitozanom doprinelo je sporijim profilima otpuštanja (Belščak-Cvitanović et al., 2011).

Trop borovnice je još jedan interesantan izvor antocijana. Tehnika sprej sušenja se može primeniti za inkapsulaciju ekstrakta tropa borovnice u izolat proteina surutke. Analiza fizičko-hemijskih svojstava inkapsulata tokom skladištenja pod različitim uslovima ukazala je na potencijal primene pripremljenih prahova kao sastojka funkcionalnih prehrambenih proizvoda (Flores et al., 2014).

Pored toga, ekstrakt tropa borovnice se može inkapsulisati primenom tehnike sprej sušenja i u matriks pripremljen od proteina surutke i pektina. Pokazano je da se na taj način može postići stabilnost antocijana i omogućiti njihovo otpuštanje i delovanje na ćelije kancera (Kropat et al., 2013).

Ostaci od prerade višnje su takođe bogati izvori fenolnih jedinjenja. Tako, na primer, ekstrakt tropa višnje se može inkapsulisati tehnikom liofilizacije u matriks koga čine maltodekstrin i arapska guma. Pokazano je da izbor nosača i udeo aktivna komponenta/nosač imaju veoma značajan uticaj na efikasnost inkapsulacije, morfologiju i veličinu mikrokapsula (Cilek et al., 2012).

Pored polisaharida, i proteini se mogu koristiti za inkapsulaciju ekstrakta tropa višnje metodom liofilizacije. Ovde se kao nosači mogu primeniti proteini surutke i soje. Dobijeni inkapsulati se mogu dodati u različite vrste keksa kao zamena dela brašna, što se pokazalo pozitivnim na funkcionalnu vrednost proizvoda. Naime, dodatak inkapsulata doprineo je većem sadržaju fenolnih jedinjenja i snažnijoj antioksidativnoj aktivnosti obogaćenog keksa bez negativnog uticaja na senzorne karakteristike. Pored toga, inkapsulacijom je postignuta stabilnost bioaktivnih jedinjenja tokom proizvodnje i skladištenja (Tumbas Šaponjac et al., 2016).

Još jedan interesantni izvor antioksidanasa je trop crne ribizle, koji je veoma pogodan za ekstrakciju fenolnih jedinjenja. Dalje, dobijeni ekstrakt se može inkapsulisati u maltodekstrin i inulin tehnikom sprej sušenja, što doprinosi boljoj zaštiti fenolnih jedinjenja i očuvanju antioksidativne aktivnosti tokom dugoročnog skladištenja (Bakowska-Barczak i Kolodziejczyk, 2011).

Ostaci od prerade citrusa su još jedan pogodan izvor bioaktivnih jedinjenja. Dobar primer je ekstrakt sporednih proizvoda prerade citrusa u sok, koji se dalje može inkapsulisati primenom tehnike sprej sušenja u celuloza acetat ftalat, polimer čija rastvorljivost zavisi od pH vrednosti. Na ovaj način se dobijaju inkapsulati otporni na uslove u gastrointestinalnom traktu. Inkapsulacija je u ovom slučaju doprinela boljoj stabilnosti i očuvanju sadržaja fenolnih jedinjenja, antioksidativnog kapaciteta i inhibitornog efekta na metaloproteinaze (Lauro et al., 2015).

Pored citrusa, i drugo mediteransko voće, poput nara, pogodno je za dobijanje novih, biološki aktivnih proizvoda. Semenke nara se mogu koristiti za ekstrakciju ulja, koje se potom dalje može inkapsulisati tehnikom sprej sušenja u obrano mleko u prahu. Optimizacijom procesa inkapsulacije postignuta je veoma visoka efikasnost inkapsulacije (95,6%) (Goula i Adamopoulos, 2012).

Pored semenki, i kora nara se može dodatno iskoristiti, posebno za dobijanje ekstrakata bogatih fenolnim jedinjenjima. Ovi ekstrakti se dalje mogu inkapsulisati primenom tehnike sprej sušenja. U tom pogledu ispitani su različiti procesni parametri i nosači, odnosno njihove kombinacije (maltodekstrin, obrano mleko u prahu, maltodekstrin/obrano mleko u prahu, maltodekstrin/izolat proteina surutke i maltodekstrin/arapska guma). Dodatak tako dobijenih inkapsulata u pastu lešnika se pokazao kao veoma efikasan za inhibiciju lipidne oksidacije i povećanje roka trajanja paste (Kaderides et al., 2015). Çam et al. (2014) su ispitivali inkapsulaciju ekstrakata kore nara u maltodekstrin. Prema istim autorima, inkapsulacija je doprinela očuvanju sadržaja fenolnih jedinjenja. Pripremljeni inkapsulati su dodati u sladoled, što je doprinelo poboljšanju funkcionalnih svojstava sladoleda, pre svega njegove antioksidativne aktivnosti, a rezultati senzorne analize ukazali su na prihvatljivost proizvoda.

Dud se takođe može koristiti kao dobra sirovina za dobijanje ekstrakata bogatih antioksidansima. Jedan od načina inkapsulacije ekstrakta duda obuhvata njegovu disperziju u lipozomima, koji se potom oblažu hitozanom i mešaju sa rastvorom maltodekstrina pre procesa sprej sušenja. Pripremljeni prahovi lipozoma sa inkapsulisanim ekstraktom duda korišćeni su za proizvodnju crne čokolade, pri čemu su ispitane različite temperature končiranja i pH uslovi. Pokazano je da je inkapsulacija u lipozome obložene hitozanom doprinela zaštiti antocijana pri dejstvu više temperature i alkalne sredine, kao i očuvanju njihove *in vitro* biodostupnosti (Gültekin-Özgüven et al., 2016).

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ENCAPSULATION OF BIOACTIVE COMPOUNDS DERIVED FROM FRUIT PROCESSING BY-PRODUCTS

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A b s t r a c t

An increased environmental awareness has led to new trends in food industry, which are reflected in intensive studies on exploitation of fruit processing by-products. Additionally, consumers' tendency to a healthy lifestyle has initiated the development of diverse functional food products. High amounts of by-products, such as peels, seeds, and stones, are discarded during fruit processing. It represents a problem both from the environmental and the economic point of view. On the other hand, the resulting residues are potential sources of numerous bioactive compounds. Therefore, fruit processing by-products such as substrates for the extraction of phenolic compounds, natural pigments, dietary fibers, protein isolates and oils attract great interest. These extracts have a great potential for the development of dietary supplements and new functional food products with beneficial health effects. However, bioactive compounds are susceptible to degradation, which represents a critical factor for their successful incorporation into food products. In this regard, the main challenge is to ensure the stability of bioactive compounds during processing, storage and in the gastrointestinal tract, i.e. to preserve their bioactivity and bioavailability. This challenge could be accomplished by the use of encapsulation. Namely, the formation of a physical barrier between an active compound and its surrounding is an effective way of protection. The present paper indicates the potential of by-products originating from the processing of apples, grapes, plums, raspberries and sour cherries as sources of bioactive compounds. It also points out the benefits that could be achieved by the encapsulation of bioactive compounds extracted from fruit processing by-products in order to develop new functional food products.

Key words: fruit processing by-products, encapsulation, bioactive compounds, apple, grape, plum, raspberry, sour cherry.

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SOIL MOISTURE INDUCED GENOTYPE-BY-ENVIRONMENT INTERACTION FOR ROOT VOLUME OF UPLAND RICE

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Abstract: Sixteen rice genotypes comprising established cultivars, recent releases and breeding lines were established in the greenhouse under different moisture levels, obtained from a combination of the amount and number of times of moisture application, to study genotype-by-environment interaction (GEI) for root volume (RV), and also probe into the level of moisture imposition, that would be adequate for screening of genotypes for response to soil moisture stress. Across the simulated environments, WAB 880-9-32-1-1-12-HB had the largest root volume of 8.71 cm^3, whereas ITA 257 had the lowest (4.89 cm^3). Genotype (G) accounted for significant ($P < 0.001$) 10.6%, environment (E) ($P < 0.001$) captured 79.0%, and GEI ($P < 0.001$) 10.4% of the total sum of squares. The GGE biplot captured 82% of the G+GE and clustered the environments into two groups, with OS 6 being the best for RV in the rainfed environment (E10). WAB 880-9-32-1-1-12-HB recorded the best RV under environments with adequate to limited moisture, but was less stable, and recorded grain production (13.5 g/plant) close to the best mean of 16.0g/plant by ITA 150 and 14.1 g/plant by IRAT 170. Environments were generally positively correlated with vegetative and yield traits, but E2 (100% moisture requirement applied once in two weeks) was more representative of the screening condition while E10 (rainfed) was highly discriminating, and would be appropriate for discarding genotypes with poor RV. Overall, E1, E2, E4 and E7 were identified as moisture conditions that are appropriate for selection of genotypes for general adaptation for RV within the overall goal of developing drought tolerant rice.

Key words: GGE, drought tolerance, grain yield, *Oryza sativa*, *Oryza glaberrima*, stability.

Introduction

Rooting and related traits in rice have received some amount of attention in the development of drought tolerant genotypes of rice. The importance of root

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traits for drought adaptation is premised on the ability to scout for soil moisture-based nutrient solution under drought conditions which occur at different stages of growth (Kamoshita et al., 2008). Water stress can reduce grain filling to zero, and can significantly reduce harvest index (Boonjung and Fukai, 1996; Bouman et al., 2006; Kato et al., 2006). Fukai and Cooper (1995) had discussed the importance of deep roots to improved water uptake under drought conditions. Wang et al. (2009) observed a marked reduction in root length (nodal and lateral) as a consequence of drought. Conversely, a progressive increase in root length and depth with the onset of drought leads to root modifications: shoot biomass partitioning as reported by Price et al. (2002) and Asch et al. (2005). Improvement of a large and deep root system of rice in order to capture more soil solution, especially under drought, would stabilise grain yield (Kondo et al., 2003).

Differences in genotypes for root density and ability to surmount soil resistance were reported by Cairns et al. (2009) although a significant interaction of site and root density was not observed. Price et al. (2002) observed a significant interaction of the type of drought and influence of year on root length and root thickness in the F_6 population, but not consequently on the two established lines, exposed to different drought simulations. Kondo et al. (2003) also reported a significant genotype-environment interaction for root dry weight under different soil conditions, and in addition, differences in the structure of genotype-environment interaction for root traits. The difference in root architecture which translated to the differential ability of genotypes to utilise soil moisture, and hence exhibited drought tolerance, was reported by Wang et al. (2009). Nassir and Adewusi (2015) reported differences in the pattern of response of root traits of rice to drought across upland soil moisture conditions. Whilst genotype responses in terms of root volume and root fresh weight appeared to be similar in their reaction to moisture limitation, the reaction and classification of genotypes were markedly different for root thickness and branching. Root volume is undoubtedly described by the number of roots (itself a function of branching), root length and root thickness, as conditional for both large root system and weight.

Genetic differences in root trait biology and its implication for drought response were extensively reviewed by Gowda et al. (2011). Extensive variation in rice root anatomical and morphological traits, and in their heritability, as reported, underscores the potential for further exploration of rice populations and traits in breeding efforts for rice tolerant cultivars. Further efforts to improve available drought tolerant rice cultivars have led to the expansion of the interspecific hybrid populations from *Oryza sativa* and *O. glaberrima* cross (Africa Rice Center [WARDA]/FAO/SAA, 2008). Selections that vary in a number of agronomic traits have been made, some of which have been released for cultivation in the upland ecology. The upland ecology is, however, characterised by variation in soil water within the growing season and this is occasioned by inconsistency in rainfall, both

magnitude and spread. Some of the traits specific to the upland ecology include earliness, tallness to compete favourably with weeds, resistance to blast, insects and lodging, good tillering erect leaf angle, compact and well exserted panicles. Obviously, rice populations are in a constant flux in terms of their genetic traits and their adaptations, especially to water-limited environments.

An assessment of genotype and cultivation (test) environment is one of the features of genotype-by-environment analysis of root traits using the biplot method (Gauch, 2006; Yan et al., 2000; 2007). The method has proved useful in grouping environments and eliminating redundant ones. The range of moisture stress encountered on the field and the cumulative effect of the adequate-inadequate moisture flux on root volume, as a function of the plant ability to survive drought, require investigation by using variable genotypes. The moisture difference is, however, a major contributor to the location effect on crop performance in the tropics. Kato et al. (2006) have affirmed that yield stability under variable soil moisture regimes represents a putative plant attribute for developing superior upland varieties. The aims of this research were to: 1) use disparity in water availability to rice genotypes to create different water-based 'environments' and examine the response of rice genotypes in terms of root volume differences under variable moisture conditions; 2) assess the stability of rice genotypes for root volume in ten moisture-based environments; 3) show the adaptability and heritability of rice genotypes in terms of root volume under ten moisture-based environments, and 4) select environments with the best discriminatory ability and representativeness for root volume of rice.

Materials and Methods

Sixteen rice genotypes developed for upland cultivation were obtained from the New Africa Rice Centre at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The genotypes include NERICA releases (from interspecific *Oryza sativa* × *Oryza glaberrima* crosses) and other *Oryza sativa* selections. NERICAs 1−5 are selections from WAB 56-104 × CG 14 cross. WAB 880 series are selections from WAB 56-50 × CG 14. WAB 56-50 is from IDSA6 (Columbia) × IAC 164 (Liberia). WAB 181 and other WAB parentage are not clear but are breeding line selections from IDSA 6 and IAC 164 (Africa Rice Centre [WARDA]/FAO/SAA, 2008). The genotypes with their designation, origin and status are presented in Table 1.

The study was conducted in the screenhouse of the College of Agricultural Sciences, Olabisi Onabanjo University, Ayetoro, which is located in a derived savannah ecology of South Western Nigeria (7°14'17"N 3°2'42"E). The location had a total rainfall of 654.8 mm, mean relative humidity of 78.7% and average temperature of 29.2 ^{0}C over the four-month study duration (May–August, 2014). Three-week-old rice seedlings of the studied genotypes were transplanted in

replicates onto black polythene bags, measuring 28 cm in diameter and 28 cm in depth, previously filled with 5 kg of loam top soil, obtained from the upland paddy. All plants had adequate moisture up to two weeks after transplanting.

Table 1. Genotypes used in the study with their designation, origin and status.

Genotype	Designation	Origin	Status
WAB 880-9-32-1-1-12-HB	G1	Cote D'Ivoire	Breeding line
NERICA 1 (WAB 450-1-B-38-HB)	G2	Cote D'Ivoire	Recent release
ITA 150	G3	Nigeria	Established line
WAB 56-50	G4	Cote D'Ivoire	Breeding line
NERICA 2 (WAB 450-11-1-P31-1-HB)	G5	Cote D'Ivoire	Recent release
NERICA 3 (WAB 450-1-B-P-28-HB)	G6	Cote D'Ivoire	Recent release
WAB 224-8-HB	G7	Cote D'Ivoire	Breeding line
NERICA 4 (WAB 450-1-B-P-91-HB)	G8	Cote D'Ivoire	Recent release
ITA 321	G9	Nigeria	Established line
NERICA 5 (WAB 450-11-1-P31-HB)	G10	Cote D'Ivoire	Recent release
WAB 189-B-B-B-HB	G11	Cote D'Ivoire	Breeding line
OS6	G12	Zaire	Established line
ITA 257	G13	Nigeria	Established line
WAB 337-B-B-20-1-129	G14	Cote D'Ivoire	Breeding line
IRAT 170	G15	Nigeria	Established line
WAB 181-18	G16	Cote D'Ivoire	Breeding line

The moisture treatments were thereafter imposed following a factorial arrangement of three water regimes and three times of application, between maximum tillering and maturity, to generate moisture mediated 'environments' (E). The previous pilot study at the location over the similar period had revealed moisture requirements to average 1.6 l per plant per week between tillering and maximum tillering stage; 2.4 l per plant per week at panicle initiation stage, and 3.2 l per plant per week at grain filling stage. Moisture-based environments E1, E2, and E3 received the full amount of moisture for each stage, applied twice weekly, once weekly, and once in two weeks, respectively. Moisture-based environments E4, E5, and E6 received 75% moisture twice weekly, once weekly, and once in two weeks, respectively. E7, E8, and E9 received 50% moisture applied twice weekly, once weekly, and once in two weeks, respectively. A set of each genotype was exposed entirely to rainfall at the E10 making a total of ten treatments. The imposition of drought for the purpose of investigation of drought tolerance, with the differential amount of moisture, is consistent with the study by Kamoshita et al. (2008). The pots were arranged following the randomised complete block design with three replications.

Data collection and analysis

Data collection on the vegetative traits and yield characters (grain weight per plant) was made as described by Anon (1988). After harvesting, the remaining

plant parts were recovered by carefully washing off the soil from the roots. The shoot and the roots were separated, and used to obtain root volume, measured as the volume of displaced water when soil-free root was inserted in distilled water. The mean values of root volume were subjected to a combined analysis of variance. Genotype-by-environment analysis was done with the SAS software (Version 9.2). The additive main effect and multiplicative interaction (AMMI) and genotype and genotype-by-environment (GGE) analyses were done with the GGE biplot software following the models described by Zobel et al. (1988) and Yan et al. (2000; 2007).

Results and Discussion

Table 2 showed descriptive statistics for root volume of rice for ten environments.

Table 2. Descriptive statistics for root volume of rice for ten environments.

Environment	Label	Mean (cm^3)	Min (cm^3)	Max (cm^3)	CV (%)	Heritability
1	E1	8.1	5.1	13.0	8.41	0.938
2	E2	8.7	5.4	12.8	8.18	0.956
3	E3	3.5	1.5	5.6	15.90	0.794
4	E4	6.6	3.8	11.0	11.07	0.918
5	E5	6.9	4.3	10.9	7.17	0.959
6	E6	3.0	1.8	4.7	13.31	0.894
7	E7	4.8	2.6	10.4	13.36	0.938
8	E8	4.2	2.6	7.7	11.89	0.917
9	E9	1.9	0.3	6.1	23.76	0.951
10	E10	12.1	7.9	18.4	6.40	0.977

Environment E10, which had superfluous rainfall over the study months, recorded the largest mean root volume of 12.1 cm^3, with a range of 7.9 cm^3 to 18.4 cm^3. The lowest mean root volume of 1.9 cm^3 (with a range of 0.3–6.1 cm^3) was recorded in environment E9. Generally, a decline in root volume followed the gradient of a reduction in the volume of water and the times of application. This observation further confirms the injurious effect of within-season variation in moisture availability to rice plants, and necessitates the importance of developing varieties that can make the required adjustment such as to minimise the effect of such drought on grain yield. The decline also gives an indication of the large linear response of root volume to increasing or decreasing drought.

The coefficient of variation was generally low ranging from 6.4 for E10 to 23.76 for E9. The heritability estimates were high to very high in all environments, with the lowest of 0.794 for E3, and the highest of 0.977 for E10. By implication, the genotypes exhibited low variability in root volume within each of the environments, but the range of CV of 6.4 to 23.76 indicated large differences in root volume between environments, implying that differences in soil moisture

conditions would influence genotypic selection for root volume. The minimal inconsistency in the heritability estimates along moisture gradients is an indication of genotype-environment and soil-moisture interactions in root volume expression.

Table 3 shows the genotype means and coefficient of variation (CV) for root volume (RV) and grain weight per plant (GWPP) across all test environments. The root volume for genotypes ranged from 4.89 cm^3 for ITA 257 (G13) to 8.71 cm^3 for WAB-9-32-1-1-12-HB (G1). The genotype coefficient of variability for root volume was high to very high. Grain weight per plant (GWPP), however, had exceptionally high CV in the range from 63 for ITA 150 (G3) to 130 for ITA 321 (G9). ITA 150 also recorded the largest GWPP of 15.99 g followed by IRAT 170 (G15) with 14.09 g. The lowest GWPP (7.37 g) was obtained for WAB 224-8-HB (G7). Root volume and grain weight per plant were significantly ($P < 0.01$) correlated ($r = 0.789$), showing the opportunity for increasing both simultaneously. The relatively higher CV for GWPP by genotypes across environments points to the possibility of further selection for root volume and grain output. The significant positive correlation of the two traits would be an added advantage. Although the ability to produce deeper and thicker roots for the extraction of soil solution is a desirable feature (Fukai and Cooper, 1995; Kondo et al., 2003), the ability to scout for moisture with more soil-root contact, as mediated by higher root volume under challenging moisture conditions, would be quite important. Obviously, the genotype ability to scout for water, based on different root sizes and architectures (Wang et al., 2009), is expressed here with the differences in root volume.

Table 4 presents AMMI analysis of variance for root volume of upland rice genotypes under different moisture-based environments. Genotype (G), environment (E) and G × E components were highly significant ($P < 0.001$), each accounting for 10.6%, 79%, and 10.4%, respectively, of the total sum of squares. The IPCA1 captured more than 50% of the G × E, with the first three axes jointly harbouring 81.3% of the interaction. The genotype and genotype-by-environment interaction (G and G x E) were responsible for 21% of the differential root volume observed.

The response to the environment was expectedly large, and normally would mitigate an equally large linear response of genotypes. The differences in genotypic and environmental proportion of G × E interaction for root traits have been reported by Kondo et al. (2003). The G × E was, however, considerable enough to necessitate a further analysis in order to identify genotypes that were both high performers and stable for root volume under different soil moisture conditions. The significant proportion of IPCA1 and IPCA2 to G × E interaction further alludes to the presence of both crossover and non-crossover interactions (Yan et al., 2007), and suggests the need for growing rice genotypes in multiple environments, in order to provide adequate conditions for genotype selection for root volume.

Table 3. Genotype means and coefficient of variation (CV) for root volume and grain weight per plant across all test environments.

Genotype	Label	Root volume (cm^3)	CV (%)	Grain weight per plant (g)	CV (%)
WAB 880-9-32-1-1-12-HB	G1	8.71^{a}	38	13.53^{b}	85
NERICA 1 (WAB 450-1-B-38-HB)	G2	7.02^{c}	63	12.21^{c}	70
ITA 150	G3	5.12^{fg}	54	15.99^{a}	63
WAB 56-50	G4	4.91^{g}	53	8.02^{gh}	100
NERICA 2 (WAB 450-11-1-P31-1-HB)	G5	4.95^{g}	45	7.80^{gh}	106
NERICA 3 (WAB 450-1-B-P-28-HB)	G6	7.47^{b}	58	9.02^{efg}	75
WAB 224-8-HB	G7	5.64^{e}	47	7.37^{h}	93
NERICA 4 (WAB 450-1-B-P-91-HB)	G8	5.55^{e}	57	8.93^{efg}	66
ITA 321	G9	7.54^{b}	53	8.50^{fgh}	130
NERICA 5 (WAB 450-11-1-P31-HB)	G10	5.33^{ef}	44	10.46^{d}	67
WAB 189-B-B-B-HB	G11	5.38^{ef}	53	8.32^{fgh}	90
OS6	G12	6.06^{d}	73	9.94^{de}	103
ITA 257	G13	4.89^{g}	66	9.66^{def}	65
WAB 337-B-B-20-1-129	G14	5.52^{e}	52	11.95^{c}	87
IRAT 170	G15	5.58^{e}	55	14.09^{b}	103
WAB 181-18	G16	6.18^{d}	58	12.20^{c}	101

a,b,c…are Duncan's multiple range test (DMRT) values. Means marked with the similar alphabets are not significantly different from one another.

Table 4.AMMI analysis of variance for root volume of upland rice genotypes under different moisture-based environments.

Source	df	SS	MS	SS (%)	GE (%)
Total	479	5433.391			
Genotype	15	564.8018	37.653***	10.6	
Environments	9	4195.079	466.120***	79.0	
G x E	135	550.67	4.079***	10.4	
IPCA1	23	291	12.65***		52.8
IPCA2	21	101	4.79***		18.3
IPCA3	19	56	2.94***		10.2
IPCA4	17	48	2.81***		8.7
IPCA5	15	33	2.19***		6.0
IPCA6	13	12	0.93***		2.2
Residual	27	10	0.38***		1.8
BLK(ENV)	20	11.95292	0.597		
Error	300	110.887	0.37		

***, significant at $P < 0.001$.

The additive main effect and multiplicative interaction (AMMI) biplot is shown in Figure 1. WAB 880-9-32-1-1-12-HB (G1) had the largest root volume and also showed positive interaction with E1, E4 and E5. G8, G13 and G15 had below-average mean values for root volume and a positive interaction of these genotypes with any environment was absent. E10 recorded the highest root volume

across all genotypes, and shared positive main effects and negative IPC1 with E2, interacting with G2, G6, G9, G12, G16 as the specifically adapted genotypes.

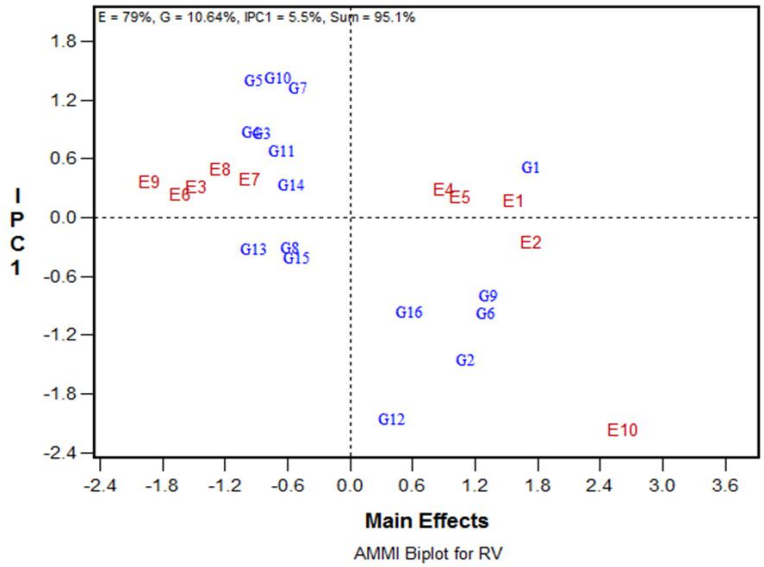

Figure 1. AMMI1 biplot of root volume of sixteen rice genotypes in ten moisture-based environments.

The other genotypes had the below-average mean root volume, but positive IPC1 scores, and were favourites in poor moisture environments represented by E3, E6, E7, E8 and E9.

The AMMI biplot separated genotypes based on mean root volume and interaction with environment. Genotypes with positive IPCA1 are expected to improve as the cultivation environment becomes better, whilst those with negative IPCA1 would do otherwise (Zobel et al., 1988; Samonte et al., 2005). Obviously, none of the genotypes had a good combination of larger and stable root volume, even though G1 appeared more acceptable, especially when the drought was not extreme, as depicted in environments E1, E5 and E4. The underlying necessity for a combination of good mean performance and stability for root volume is further made obvious.

The genotype and genotype × environment (GGE) biplot for root volume of sixteen rice genotypes in ten moisture-based environmentscaptured 82% of the GGE (Figure 2), and classified the environments into two groups. An attempt to separate groups for analysis did not provide any meaningful gain in the genotype and environment classification; hence all environments were included in the analysis. In

the group comprising all environments except E10, G1 was the superior genotype, whereas G6 and G9 were also in the sector. In environment E10, G12 was the best genotype regarding root volume, but with G8, G15 and G16 sharing this polygon sector. The genotypes in the sector, however, had low root volume, but G15, which had the lowest root volume, produced the second best grains after G1.

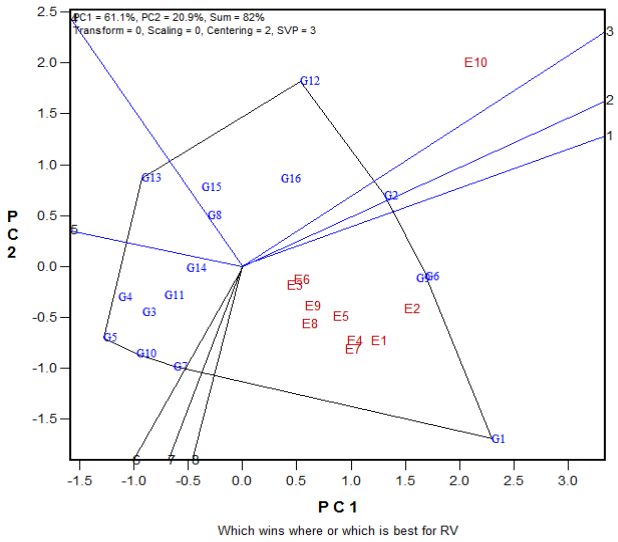

Figure 2. The genotype and genotype × environment (GGE) biplot for root volume of sixteen rice genotypes in ten moisture-based environments.

The G1 sector comprised environments with the highest to lowest amount of moisture, and those with the adequate to moderate amount of moisture (and by extension, moderate drought). Going by the underlying principle of the which-wins-where attribute of the GGE biplot (Yan et al., 2001; 2007), G1 appeared to be capable of above-average rooting under these moisture conditions. The PC1 of the GGE correlated significantly ($P < 0.01$, data not shown) with fresh root weight (0.972), fresh shoot weight (0.629), total fresh weight (0.774), indicating that the root volume performance also translated to higher values for other rice plant traits. In addition, root volume was significantly positively correlated ($P < 0.01$) with all vegetative and yield traits, with the highest correlation of 0.990 observed with grain weight per plant, while the lowest correlation of 0.775 was found with spikelet fertility. Genotypes G1 and G6 produced fewer grains on the average, compared to G3, and would probably benefit from introgression of gene from G3 for higher grain output. G12, G16, G15 and G8 would give higher root volume under higher moisture conditions typical of E10.

The mean versus stability view of GGE for root volume is displayed in Figure 3. Expectedly, G1 was identified as having the best root volume and was also less stable though better in this regard than most of the genotypes. G14 was the most stable but with below-average mean root volume compared to G1.

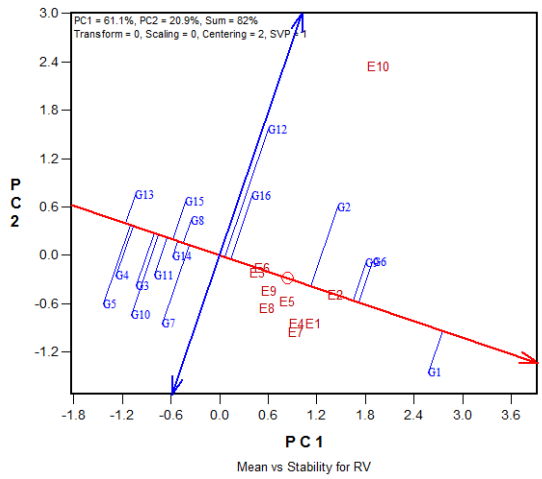

Figure 3. Genotype mean and stability for root volume of sixteen upland rice genotypes in ten moisture-based environments.

The ranking of genotypes relative to G3, an established upland cultivar with a record of good grain yield (Figure 4), and also an ideal genotype with both high mean performance and high stability across environments, confirms the above order of the genotypes in terms of mean performance. Only G14 was more stable than G1. Conversely, G12 was the most unstable, and thereby confirmed its inclusion in the E10 sector of the which-wins-where biplot. G1 had better stability compared to G3 and also better root volume. With introgression of traits for improved grain production, it may replace G3 in the ecology.

By these observations, questions may be raised as to the possibility of some compensatory relationship between root volume and grain yield (Nassir and Ariyo, 2006). Although there was a significant positive relationship between root volume and grain yield indices in this study, the increased concentration of genes that consolidated the traits would further narrow the possible counteracting expression of the traits. Nonetheless, G1 and G14 merit further studies specifically for their use in developing varieties with a good combination of larger and stable root volume, on the one hand, and grain productivity on the other.

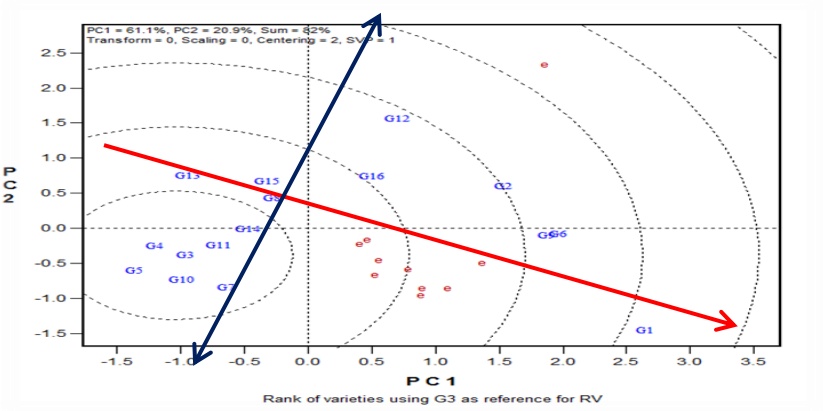

Figure 4. The genotypic relationship and ranking with ITA 150 (G3) as a reference.

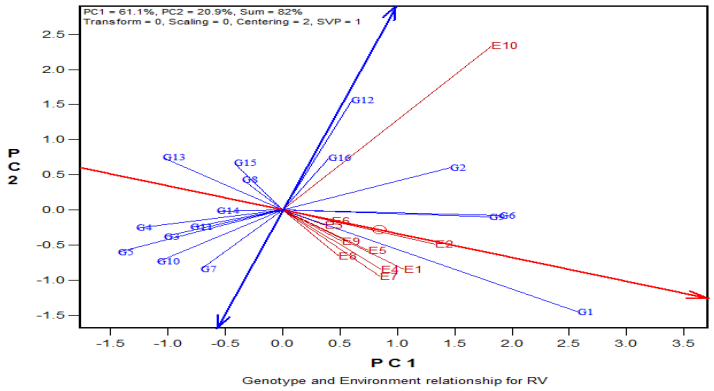

Figure 5. The discriminating ability vs. representativeness of the moisture environment for root volume of rice.

The discriminating ability vs. representativeness for root volume of rice is exhibited in Figure 5. The view allows the classification of the environments by their discriminating power, eliminating redundant test environments, and categorising their representativeness in evaluating genotypes based on the underlying objective (Yan et al., 2007). Environment subgroup 1 comprised all environments except E10 which is the only entry in subgroup 2. E10 had a combination of the long vector and large angle with the average environment coordinate (AEC) axis and was on the opposite side of the AEC line compared to subgroup 1. Subgroup 1 caused genotypes to have some of the poorest root volume in contrast to subgroup 2. The environments in subgroup 1 had short- to medium-length vectors, but relatively smaller angles with AEC, with E6, E2 and E3, thereby having the bestrepresentativeness in that order.

E2, however, appeared to be the best of the three by virtue of having the longest vector while E3 and E6 with short vectors may not elicit distinct differences between the genotypes.

Environment 10 was obviously the most discriminating of the genotypes for root volume, whilst E2 was more representative of moisture-based environment for screening of rice genotypes for root volume. Clearly, there were duplications which necessitate dropping of some of the environments to save cost, and make the experiment more efficient and analysis more concise. For instance, E1 (full moisture, applied twice weekly), E2 (full moisture applied once weekly), E4 (75% moisture applied twice weekly) and E7 (50% moisture applied twice weekly) can be used while others (E3, E5, E6, E8 and E9) can be discarded without any loss in interpretation and decision making.

Conclusion

Variable soil moisture regimes created by a combination of the amount and times of water application to rice genotypes created different moisture environments that elicited both proportional and disproportional differences in root volume, hence the significant genotype-by-environment interaction. Studied genotypes expressed differences in root volume under the different moisture conditions, and offered insight into their use for development of genotypes with a stable response to root formation, and eventual plant performance under poor soil moisture conditions. The largest root volume was recorded in the rainfed environment and declined with reducing moisture. WAB 880-9-32-1-1-12-HB (G1) had the largest root volume of 8.71 cm^3, recorded the third best grain production, but was less stable compared to WAB 337-B-B-20-1-129 (G14) which had low root volume. AMMI analysis captured 95.1% of the total variation but did not identify any genotype as having a combination of large and stable root volume. The GGE biplot summarised 82% of the G+GE and identified WAB 880-9-32-1-1-12-HB (G1) as the best genotype for root volume in environments with adequate to limited moisture. E10 (rainfed) was very discriminatory and would be appropriate for identifying unstable genotypes while E1 (full amount of moisture for each stage, applied twice weekly), E2 (full amount of moisture for each stage, applied once weekly), E4 (75% moisture applied twice weekly) and E7 (50% moisture applied twice weekly) (all with adequate to limited moisture) were more representative of screening environments for rice root volume.

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INTERAKCIJA GENOTIPA I SPOLJAŠNJE SREDINE INDUKOVANA VLAGOM ZEMLJIŠTA ZA ZAPREMINU KORENA BRDSKOG PIRINČA

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R e z i m e

Šesnaest genotipova pirinča koji su obuhvatali komercijalne sorte, nedavno priznate sorte i oplemenjivačke linije gajeno je u stakleniku pri različitim nivoima vlage, dobijenih kombinacijom količine vode i broja zalivanja. Cilj ispitivanja je bio interakcija genotipa i spoljašnje sredine (engl. *genotype-by-environment interaction*− GEI) za zapreminu korena (engl. *root volume*− RV), kako bi se utvrdio nivo vlage, koji bi bio adekvatan za ispitivanje genotipova za odgovor na stres izazvan vlagom. U simuliranim spoljašnjim sredinama, WAB 880-9-32-1-1-12-HB je imao najveću zapreminu korena − 8,71 cm^3, dok je ITA 257 imao najnižu (4,89 cm^3) zapreminu korena. Genotip (G) je obuhvatio značajnih ($P < 0,001$) 10,6%, spoljašnja sredina (E) ($P < 0,001$) 79,0%, a GEI ($P < 0,001$) 10,4% ukupne sume kvadrata variranja. GGE biplot je bio zasnovan na 82% od G+GE, i spoljašnje sredine su grupisane u dve grupe, sa OS 6 kao najboljim za RV u spoljašnjoj sredini sa prirodnim padavinama (E10). Za WAB 880-9-32-1-1-12-HB je utvrđen najbolji RV u sredinama sa optimalnom do ograničavajućom vlagom, ali je bio manje stabilan, i sa prinosom zrna (13,5 g/biljci) blizu najboljih proseka − 16,0 g/biljci (ITA 150) i 14,1 g/biljci (IRAT 170). Spoljašnje sredine su bile pozitivno korelisane sa vegetativnim osobinama i komponentama prinosa, ali je E2 (100% potreba za vlagom primenjena jednom u dve nedelje) bila reprezentativnija za uslove vlažnog stresa, dok je E10 (sredina sa prirodnim padavinama) bila veoma diskriminativna, te bi bila odgovarajuća za odbacivanje genotipova sa lošim RV. E1, E2, E4 i E7 su prepoznati kao nivoi vlage koji su prikladni za odabir genotipova za opštu adaptaciju za RV u okviru sveukupnog cilja oplemenjivanja pirinča tolerantnog na sušu.

Ključne reči: GGE, tolerantnost na sušu, prinos zrna, *Oryza sativa*, *Oryza glaberrima*, stabilnost.

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EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION ON SOIL PROPERTIES AND YIELD OF SELECTED RICE VARIETIES

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Abstract: Plant growth can be stimulated by a symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and bacteria within the rhizosphere region. These interactions are crucial for increasing soil fertility, which leads to increased productivity and sustainability, as well as food security considering a high level of malnutrition. Six rice varieties were grown with (M+) or without (M-) AMF inoculation in a randomised complete block design with three replicates. The soil physic-chemical properties were determined using standard procedures. Bacteria were isolated from the soil samples and the colony count was determined during the early and late cropping seasons of rice. Specific soil properties (phosphate, pH, organic matter) increased dramatically in the presence of AMF, which led to significant rice yield in both seasons. Bacterial species isolated included *Lactobacillus* spp., *Klebsiella aerogenes, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens*, *Azospirillum brasilense, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae,* and *Micrococcus* sp. Rice exudates increased the bacterial population in the early season, while AMF treatment increased the bacterial population in the late season and generally increased the bacterial species richness in both seasons. Although the actual mechanism that increased the bacterial species richness was not accessed, this study, however, shows that AMF-bacteria interaction increased and sustained soil fertility which consequently increased rice yield. A further study is necessary to determine the mechanism of the interaction observed between AMF inoculation and bacterial population.

Key words: mycorrhizal, phenolic compounds, NERICA, colonisation.

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Introduction

Rice is the most widely consumed staple food for a large part of the world's human population, especially in Asia and the West Indies. Since a large portion of maize crops are grown for purposes other than human consumption, rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by the humans. It is predicted that a 50% to 60% increase in rice production will be required to meet demand from population growth by 2025 (Zhang and Wang, 2005). Interestingly, sub-Sahara Africa has the greatest concentration of high-value minerals and the highest concentration of degraded soils. It has the fastest growth in agriculture and the greatest level of agricultural imports. Sub-Sahara Africa has the highest proportion of the rural and poor smallholders in agriculture notwithstanding it has the fastest growth in agriculture and the greatest level of agricultural imports (Livingston et al., 2011). An ever increasing population and lifestyle have contributed to a demand for rice within the region making it the most imported food commodity (Livingston et al., 2011). Having huge resources for rice production, most African governments have put in a great deal of efforts to develop the local rice sector as an important component of national food security, economic growth, and poverty alleviation which has been receiving great attention but is still confronted with low yield (Balasubramanian et al., 2007; FAOSTAT, 2015).

Several challenges have been encountered by numerous local farmers, which include: low soil fertility, biodiversity, poor management, climate change and policy issues. These challenges have immensely affected the productive capacity of rice within Nigeria and sub-Sahara Africa to meet the demands of growing populace, hence research and development preceded the development of rice breeds capable to withstand disease, pests, drought and soil infertility and low yield. In so doing, rice breeders utilised biotechnology to perform cross-fertilisation and back-crossing between rice species *Oryza sativa* and *Oryza glaberrima* which are the Asian and African historic species of rice, respectively to produce several traits of the 'New Rice for Africa' (NERICA) (West Africa Rice Development Association, 2001). These strains of rice happened to be effective in suppressing weeds, drought tolerant, resistant to pests and diseases, mature early, have favoured qualities, more grains per panicle, non-shattering grains and higher protein content. With such rice species and strains, there is the potential for Nigeria and other counterpart countries to be rice self-sufficient and when effectively managed with government support, to upgrade to become exporters of rice to the globe.

There would be a prolific implementation of the hybrid rice species, hence significant knowledge is required to understand what occurs below the ground where microorganisms within the rhizosphere interact with the rice species through

the root via the symbiotic relationship. The arbuscular mycorrhizal fungi (AMF) represent a vital component of the majority of all terrestrial ecosystems. They form a symbiotic relationship with the roots of most plants by increasing plant phosphate (P) uptake and growth, whilst the plants supply the AM fungi with exudates for metabolism (Smith et al., 2003). Seal et al. (2004) quantified common compounds secreted by the rice plants within the rhizosphere which include: thiol acid, phenol, phenolic acid, indole, terpenic acid and phenylalkanoic acids. Transgenic rice species possessing proteins that confer resistance to pests have been shown to release root exudes containing the said protein pesticide into the soil microenvironment (Saxena et al., 2004). In return, AMF possess distinct mycelium that provides a pathway for translocation of photosynthetic-derived carbon to soil microenvironments. This happens when there is a rapid turnover of the mycelium and exudation (glucose, starch, oligosaccharides) of living hyphae can enhance bacterial growth and composition within the soil (Staddon et al., 2003). An increased knowledge of the responses of bacteria composition and growth in the presence of AMF during cultivation of the newly developed rice species in soil is required to understand the effects on soil properties. The objectives of this study were to investigate (i) the effects of AMF treatment on soil properties and plant yield in soils containing different species of rice and (ii) the effect of AMF treatment on the indigenous bacterial population and composition within the rhizosphere of rice varieties.

Materials and Methods

Description of experimental site and sample collection

The experiment was conducted at the upland section of the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria. The early season trial was conducted between May 26th and September 4th, 2012, while the late season planting was done between September 8th and December 8th, 2012. The site is located between the rainforest and derived savannah agroecological zone of Nigeria (7°9′ 38.9″ N lat., and 3°21′ 53.9″ E long.; 140 m a.s.l). The rice experiment was grown twice in a year during the early and late season rainfall, respectively. The experiment comprised twelve treatments which were combinations of six rice varieties with (M+) or without (M-) arbuscular mycorrhizal fungi (AMF) inoculation. Random soil samples were taken from the field before planting to determine the soil physical and chemical properties. In addition, random soil samples were collected from the rhizospheric region in each plot for the determination of bacterial count in the laboratory.

Experimental design and treatment

Each experiment was a 6 x 2 factorial experiment in a randomised complete block design with three replications. Six varieties of rice (Moroberekan, NERICA 1, NERICA 2, NERICA 3, NERICA 4, and WAB 56-104) were combined with (M+) and without (M-) AMF treatment. The AMF inoculum was obtained from the Department of Plant Physiology and Crop Production of the Federal University of Agriculture, Abeokuta. The pure inocula (*Funneliforms* sp.) were isolated and enriched from rice fields across Nigeria, identified as *Glomus mosseae* and *Glomus geosporum* and mixed according to Brundrett (2004). The mixed inocula were then applied to the hole (50 g per hole) made for rice seeds to be planted and referred to as the AMF treated (M+) plot, and the plots containing the rice variety without the AMF treatment were referred to as the AMF non-treated plots (M-). Rice was then planted at a spacing of 20 cm x 20 cm by the dibbling method on the plot of 4 m x 3 m (12 m^2) giving rise to 250,000 plants/ha at the rate of 3 seeds/hole.

Five plants were randomly selected from the net plot for the determination of major agronomic parameters (growth, yield and yield components). Plant height, number of tillers and dry matter accumulation were recorded at 4, 8 and 12 weeks after planting (WAP). Days to 50% flowering and 90% maturity were also determined. The number of panicles per square meter was determined with the aid of quadrant (one meter square) by counting the number of panicles in the quadrant three weeks prior to harvest (Sakariyawo et al., 2012). Other yield component parameters were determined by standard procedures (Sakariyawo et al., 2012).

Soil analysis

Soil samples were collected from the individually treated soils (before planting, M+, M-) before and after harvest for analysis. Soil properties including pH, organic carbon and organic matter contents (%), N (%), P, Mg, Ca, and K (mg kg^{-1}) were determined. Briefly, soil pH was determined in water (1:2.5) and KCl solution (1:1) using the glass electrode pH meter. The soil organic matter was determined according to the Walkey and Black (1934) method. The determination of total nitrogen (N) in the soil was analysed using the Kjeldahl method (Bremner, 1960), whilst the available phosphorus (P) was extracted using the Olsen's extract and P in the extract was determined by using a spectrophotometer. The exchangeable cations (Mg, Ca, K) were extracted with 1 N ammonium acetate, K in the extract was determined by flame photometry, whilst Ca and Mg were determined using an atomic absorption spectrometer (AAS). The QA/QC was ensured by replicate digestion, use of blanks and high percentage recovery of elements.

Bacterial isolation, identification and count

A serial dilution technique was employed on samples obtained by collecting soils attached to the roots. Six-fold serial dilutions were prepared, and appropriate dilutions were plated on nutrient agar. Similarly, 2.8 g of nutrient agar was dissolved in 100 ml of sterile distilled water in a conical flask and corked with cotton wool and foil paper. It was sterilised at a temperature of 121ºC for 15 minutes in an autoclave. After autoclaving, it was allowed to cool down at a hot to touch temperature before using it for culturing. After culturing, biochemical identification of the isolates was done, in which each organism was identified according to the Cowan and Steel method of bacteria identification (Barrow, 2003), by their colonial appearance such as size, shape, consistency, colour, elevation, and Gram staining was done to further identify the isolate. The following biochemical tests were performed to further characterise the isolates through: sugar fermentation test**,** oxidase test**,** catalase test**,** citrate utilisation test, urease test, methyl red test, coagulase test and indole test.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using PROC GLM of Statistical Analytical System package (SAS, 2001). Bacterial count data were log-transformed before analysis. Means that were significantly different at $P < 0.05$ were separated using the least significant difference (LSD).

Results and Discussion

Soil properties

The physical and chemical properties of the soils (M+ and M-) were determined prior to planting, during the early and late cropping seasons (Table 1). The early cropping season was associated with more frequent rainfall than the late cropping season (Figure 1). It was observed that the soil was originally slightly acidic prior to planting and during the cropping season in the absence of AMF inoculation. During the late cropping season, the acidity of the soil increased, which resulted in a lower pH condition than the initial state. However, the addition of AMF into the soil resulted in a marked increase ($P < 0.01$) in pH to the neutral condition during the early and late cropping seasons, which resulted in improved soil condition. Similarly, the organic matter content of the soil was initially 2.88% and further decreased at the late cropping season (2.34%), but the addition of AMF resulted in a subsequent increase in OM content owing to AMF biomass. Although OM decreased at the late cropping season, the reduction was higher in AMF-

treated soils. The concentration of macronutrients (N, Mg, K, Ca) within the soil seemed to be higher in AMF-treated soils compared to AMF-untreated soils, but was not significant ($P > 0.05$). However, in regards to P concentration in soil, the planting of rice led to a significant ($P < 0.05$) reduction in the amount of P owing to utilisation of P by the rice plant. Noticeably, there was a remarkable increase ($P < 0.001$) in concentration of P within the soil that was AMF-treated compared to untreated soils in both early and late cropping seasons. Unsurprisingly, a minimum of 35% P loss (abiotic + biotic) of the initial concentration of the available P was discovered following AMF treatment.

Table 1. Chemical properties of the experimental site.

Soil treatments	pH	Organic carbon	Organic matter (%)	Total nitrogen (%)	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})	Magnesium (mg kg^{-1})	Calcium (mg kg^{-1})
Before planting	5.7	1.55	2.88	0.13	33.22	0.26	1.90	16.10
Early season cropping after the harvest of rice								
-AMF	5.8	1.86	2.57	0.15	28.45	0.18	1.10	15.40
+AMF	7.4	2.10	3.29	0.19	195.79	0.24	2.00	15.30
Late season cropping after the harvest of rice								
-AMF	5.2	1.11	2.34	0.12	22.84	0.11	1.30	12.50
+AMF	7.2	1.99	3.06	0.18	125.32	0.19	2.00	12.40

Note: -AMF means without AMF; +AMF means with AMF.

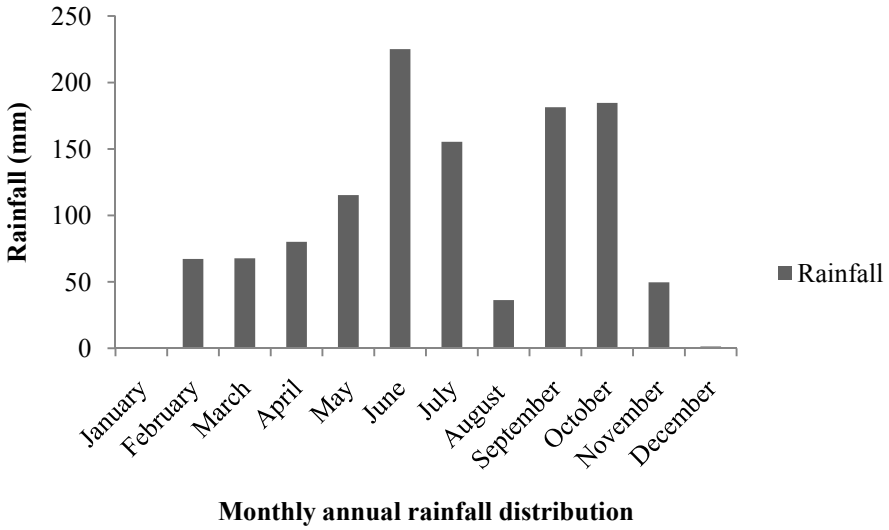

Figure 1. Total rainfall distribution in 2012.

Rice yield

It was observed that all the growth and yield parameters were significantly affected ($P < 0.05$) by the treatment both in early and late cropping seasons (Table 2). In the early season, application of AMF significantly shortened ($P < 0.05$) the number of days to 50% flowering and days to 95% physiological maturity. Moreover, panicle length, panicle weight, number of seeds per panicle and yield of rice plant with AMF were significantly higher ($P < 0.05$) than those without AMF. During the late season, the rice plant without AMF did not survive after flowering and no data could be obtained from the plots (Table 2).

Table 2. The effect of AMF inoculation on growth, yield components and yield of upland rice during early and late cropping seasons.

Soil treatments	Days to 50% flowering	Days to 95% maturity	Panicle length (cm)	Panicle weight (g)	Panicles per m^2	Seeds per panicle	100-seed weight (g)	Grain yield (kg/ha)
Early season								
- AMF	85.17	108.67	16.64	2.82	75.1	110.5	2.63	825
+ AMF	75.56	96.72	20.75	3.44	128.0	139.1	3.33	2484
SE ±	0.48	0.67	0.45	0.07	4.44	2.31	0.06	70.4
Late season								
- AMF	74.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
+ AMF	53.5	71.67	16.39	2.79	91.33	104.8	2.91	1586
SE ±	0.66	0.32	0.12	0.02	0.88	2.71	0.04	19.91

Note: -AMF means without AMF, +AMF means with AMF.

Bacterial count, composition and occurrence

The bacterial count ranged between 11×10^6 and 43×10^6 colony forming units (cfu) during the early season and from 14×10^6 to 82×10^6 cfu in the late season (Figures 2 and 3). Different bacterial counts were observed in the rhizosphere of the rice variety while AMF inoculation affected the bacterial count in both seasons (Tables 3 and 4). Different isolates were observed in AMF-treated and AMF-non-treated plots. It was also observed that in the early season, in plots treated with AMF, ten (10) different bacterial isolates were identified, while seven (7) isolates were identified in AMF-non-treated plots (Table 3). During the late cropping season, the numbers of observed bacterial isolates were different among the treated plots and non-treated plots as seven (7) different bacterial isolates were identified in both AMF-treated and non-treated plots (Table 4).

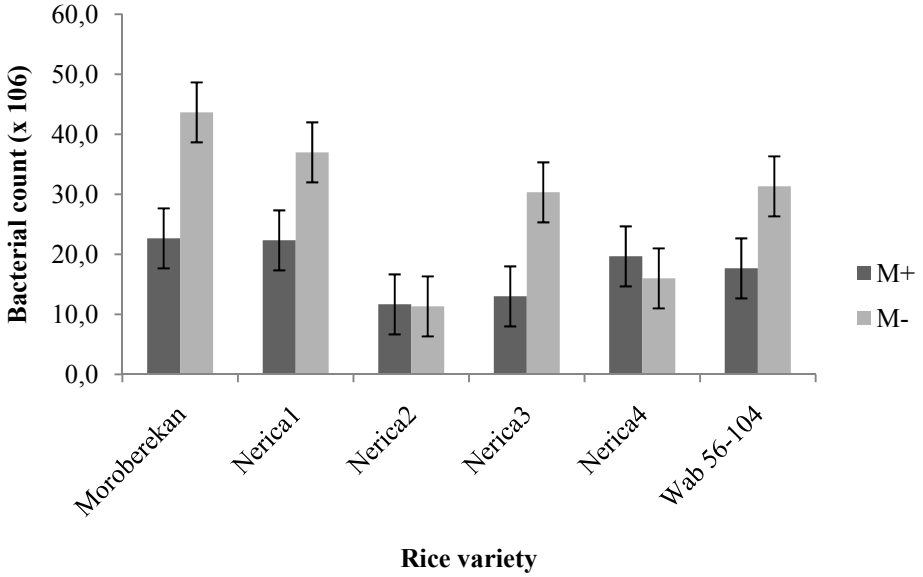

Figure 2. The effect of AMF inoculation on bacterial counts of rice in the early season.

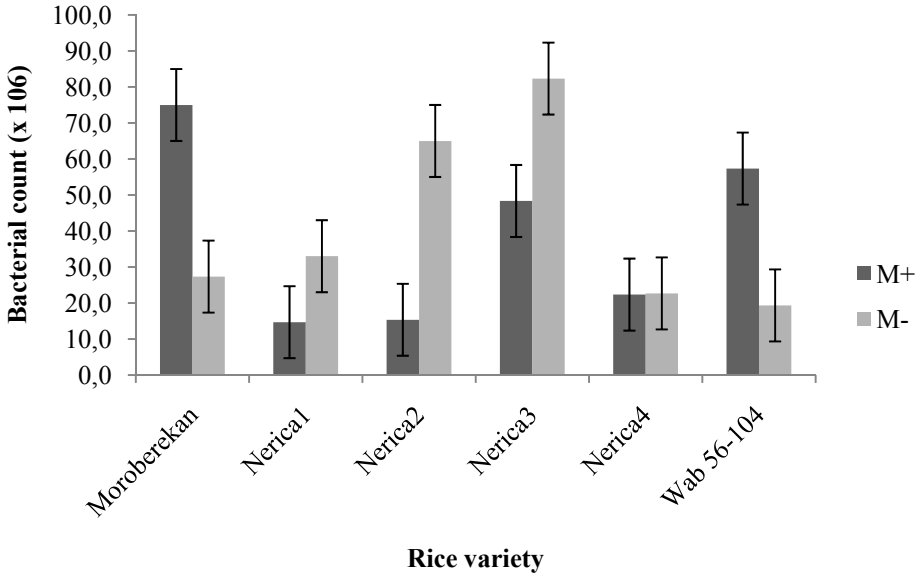

Figure 3. The effect of AMF inoculation on bacterial counts of rice in the late season.

Table 3. Bacterial isolates observed under different treatments in the early season.

Variety	With mycorrhizae	Without mycorrhizae
Nerica 1	*Lactobacillus* spp, *Micrococcus* spp, *Enterobacter cloacae*	*Bacillus subtilis*, *Escherichia coli*
Nerica 2	*Proteus mirabilis*, *Pseudomonas fluorescens*	*Proteus mirabilis, Klebsiella aerogenes, Bacillus subtilis*, *Escherichia coli*
Nerica 3	*Azospirillum brasilense, Bacillus subtilis*, *Staphylococcus aureus, Enterobacter cloacae*	*Staphylococcus aureus*, *Escherichia coli*,
Nerica 4	*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*	*Proteus mirabilis, Escherichia coli*, *Micrococcus* spp,
Moroberekan	*Enterobacter cloacae, Klebsiella aerogenes*, *Bacillus subtilis, Escherichia coli*, *Pseudomonas fluorescens*	*Escherichia coli*
WAB 56-104	*Staphylococcus aureus, Bacillus subtilis, Pseudomonas fluorescens*	*Pseudomonas fluorescens, Klebsiella aerogenes, Escherichia coli*

Table 4. Bacterial isolates observed under different treatments in the late season.

Variety	Mycorrhizae +	Mycorrhizae -
Nerica 1	*Bacillus subtilis, Pseudomonas flourescens, Enterobacter cloacae*	*Bacillus subtilis, Micrococcus* sp., *Escherichia coli, Pseudomonas fluorescens*
Nerica 2	*Bacillus subtilis, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens*	*Micrococcus* sp., *Bacillus subtilis*
Nerica 3	*Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae, Escherichia coli*	*Bacillus subtilis, Staphylococcus aureus, Escherichia coli*
Nerica 4	*Escherichia coli, Proteus mirabilis, Pseudomonas fluorescens*	*Bacillus subtilis, Proteus mirabilis*
Moroberekan	*Pseudomonas fluorescens, Escherichia coli*	*Klebsiella aerogenes, Escherichia coli*
WAB 56-104	*Staphylococcus aureus, Bacillus subtilis*	*Escherichia coli, Proteus mirabilis, Pseudomonas fluorescens*

It was realised that the frequency of bacterial strains was not consistent as some bacteria species were more associated with the presence of AMF than without AMF. For instance, *Pseudomonas* sp. was often observed to be identified in AMF treated soils during both early and late cropping seasons in plots having at least four (4) rice varieties, especially NERICA 2, 4 and Moroberekan, but they were often associated with WAB 56 variety in AMF non-treated plots. In contrast, *E. coli* was identified more in AMF non-treated plots containing NERICA 1, 3, Moroberekan and WAB 56 rice varieties, but only found consistently in the late cropping season of AMF-treated plots (Table 4).

Soil properties

The introduction of AMF into the soil was shown to increase certain soil physical and chemical properties in this study. Previous studies have shown that AMF inoculation in soils can increase the absorption of such macronutrients such as Mg and K (Singh et al., 2004; Halder et al., 2015). Although the uptake of macronutrients was not measured, it is suggested that AMF improved the availability and accessibility of these nutrients to plants. Notably, this study shows that AMF treatment drastically increased the concentration of P in the soils by releasing extracellular phosphatase enzymes (Koide and Kabir 2000; Hamel, 2004). P is a limiting factor in most agricultural soils, and this requires the incorporation of inorganic fertiliser into the soil. Similarly, Carreón-Abud et al. (2007) observed a dramatic increase in P in the aerial part of the blackberry plant (*Rubus fruticosus* var. brazos) treated with AMF. Abdel Latef and Chaoxing (2011) also observed increased P concentration and uptake into tomato when AMF was inoculated into saline soils. Even though AMF have the capability to mineralise P, Jonker et al. (2000) have reported that it is only to a minimal level. Furthermore, the fungus is capable to translocate P from the soil to the interior of the plant root system to ensure release to the plant (Smith et al., 2001; Villegas and Fortin, 2001; Toljander et al., 2007). Although P is vital for plant growth, it is immobile and plants find it difficult to access and acquire it, but accessibility is increased through a symbiotic relationship with AMF (Schachtman et al., 1998; Smith et al., 2011). More P was found in AMF treated soils in the early cropping season than in the late cropping season due to both biotic and abiotic losses (35%) via P uptake and utilisation by the plant (rice) and water erosion losses during the raining season of June and July. Unsurprisingly, despite the low concentration of P (28.45 mg kg^{-1}) in AMF non-treated soils, there was still considerable loss (20%) through biotic and abiotic uses, since uptake is normally faster than replacement (Smith et al., 2011).

In addition to improvement to soil chemical properties (macronutrients) in AMF treated soils, it was discovered that there was a significant increase in the organic matter (OM) content and pH of the soils. In line with the symbiotic relationship between AMF and interacting biota, it happens that through the hyphae, AMF release a range of compounds into the soil environment (Kögel-Knabner, 2002; Rillig 2004; Verbruggen et al., 2013) that can increase the OM content of soils. Obviously, OM contents increased considerably in AMF treated soils compared to non-treated soils. The exudate compounds released by AMF range from glucose, polysaccharides (glycogen, oligosaccharides) and organic acids to polymeric substances (gellan gum), some of which are recalcitrant and contribute to the OM content of the soil (Toljander et al., 2007; Verbruggen et al., 2013). In addition, the decomposition of plant litter has been observed to increase

in the presence of AMF (Cheng et al., 2012) and dead AMF can also add to the OM content of soils. An increase in contact time during interaction amongst AMF and soil biota and components led to a considerable loss in the OM content (7%) which was lower than OM loss (9%) in non-treated soils during the cropping seasons. This shows that the replenishment and maintenance of OM in soil containing AMF were higher than in non-treated soils, but would be governed by the soil properties, AMF exudates and climatic conditions. In regards to pH, the addition of AMF to soils increased soil pH from 5.8 to 7.4 in the early cropping season, which supports the observation of Shukla et al. (2013) that AMF species richness was higher at higher pH levels and decreased with an increase in depth and a decrease in pH. An increase in pH is suggested to be phosphate-induced, as previous studies have shown that the incorporation of P at high rates elevates soil pH and reduces concentration of toxic aluminium compounds by forming insoluble variscite-like material [Al $(OH)_2$ H_2 PO_4] precipitate in soil (Habte and Soedarjo, 1996; Manoharan, 1997). Similarly, an increase in contact time led to a decrease in the pH levels, owing to the loss of P through abiotic and biotic processes.

Rice yield

Previous studies have shown that AMF inoculation into agricultural soils improves plant growth, productivity and yield even under stress conditions (Clark and Zeto, 2000; Adesemoye et al., 2008; Zarea et al., 2009; Abdel Latef and Chaoxing, 2011). More precisely, Mäder et al. (2011) observed approximately 30% increase in wheat yield following AMF inoculation, whilst Khan and Zaidi (2007) observed over 50% higher wheat yields. However, when the AMF strain *Glomus intraradices* was inoculated to soils to investigate rice yields, Khan and Zaidi (2007) witnessed remarkable rice yield only in the second year of planting, whilst there was no significant yield earlier when compared to non-inoculated soils and they attributed it to inappropriate selection of AMF for rice. On the contrary, in this current study, there was highly significant rice yield (> 200%) during both seasons and it was highly remarkable in the later season, and this supports previous works by Fernández et al. (1997), Solaiman and Hirata (1997a) and Jhra et al. (2009).

After inoculating soil with AMF, the AMF colonise the rhizosphere region (Kaya et al., 2008) and then increase plant uptake of immobile nutrients, such as P by absorbing it from soil pool and other macronutrients (Tinker and Gildon, 1983; Bohrer et al., 2003; Abd-Alla et al., 2014), and consequently improve plant productivity. With the favourable pH condition, OM content and improved availability, uptake and translocation of P to the plant enhanced rice yield in support of Mohammed et al. (2014) and Pellegrino et al. (2015). This was also sustained in the late cropping season despite a minimal reduction in yield owing to a reduction in pH, OM and P concentration. However, the improved plant yield and

maintenance of soil nutrients by AMF are highly dependent on the level of soil salinity, drought, soil properties, climatic and environmental conditions (Fernández et al., 1997; Solaiman and Hirata, 1997b; Abdel Latef and Chaoxing, 2011; Mäder et al., 2011; Pellegrino et al., 2015).

Bacterial count, composition and occurrence

The rhizosphere (i.e., the zone of soil influenced by plant roots and characterised by vital microbiological activities) represents a highly dynamic region governed by a complex mosaic of interactions between plants and microorganisms (Kennedy and De Luna, 2004). Varietal differences can affect the communities of microorganisms living in the rhizosphere, especially when there are differences in root secretions and exudates that are released by the plant. During the early season, NERICA 2 plot without AMF inoculation had the lowest bacterial count, but during the late season, the bacterial count increased. This shows consistency with the literature on the interactions between plants and microorganisms in the rhizosphere, where the former release compounds such as carbohydrates, phenolics, acetic acids, indoles, carboxylic acids, abietic acid and amino acids (Baudoin et al., 2003; Bacilio-Jiménez et al., 2003). The concentration and composition of these compounds vary based on the rice variety, soil properties and plant response to soil conditions (Bacilio-Jiménez et al., 2003; Seal et al., 2004). These were probable reasons why microbial counts were higher in the late cropping season without AMF treatment as plants would have reduced exudation or altered exudate composition which significantly favoured certain bacterial strain growth. Hence, the composition of the exudates can be either antagonistic or stimulatory to several bacteria species during either of the cropping seasons.

Mycorrhizal treatment, on the other hand, affected the bacterial count in the first season as a higher bacterial count was observed in non-treated rice but not in treated rice varieties. Studies have shown that AMF can have a significant impact on bacterial community composition and activity through competition for inorganic nutrients and enhancement of AMF species-specific bacterial stimulation (Artursson et al., 2006). Therefore, the reduction in the bacterial count may be a result of the competition between the introduced AMF and the microorganisms in the rhizosphere or fungal exudation. However, following an increase in contact time, a striking increase in the bacterial count was observed in Moroberekan and WAB 56 AMF-treated soils which were of statistical similar population compared to the AMF non-treated NERICA 2 and NERICA 3 soils, thus suggesting a species-specific association of bacteria with AMF. This association is mainly governed by soluble factors or via physical contact between bacteria and AMF hyphae (Bianciotto et al., 1996; Artursson et al., 2006).

Rice root exudates and AMF colonisation also influenced the bacterial species composition by increasing some groups and decreasing others. For instance, during the early cropping season in AMF non-treated soils, *E. coli* was a common bacterium found in all rice varieties, but in the late cropping season it competed with *Bacillus subtilis* suggesting that the former was attracted by these rice varieties but its dominance was affected following the competition and reduction in particular rood exudates. The introduction of AMF led to an increased specific bacterial species composition and richness even though the population did not exceed that in AMF non-treated soils owing to the competition and diversity of exudates. More precisely, *Bacillus subtilis* and *Pseudomonas fluorescens* dominated in both early and late cropping seasons due to specific interactions with AMF hyphae or exudates. Previous studies have equally shown interactions (root colonisation, phosphate solubilisation, fungal growth, inhibition of plant pathogenic fungi) between *Pseudomonas* sp. and *Bacillus* sp. with *Glomus* sp. (Toro et al., 1997; Barae et al., 1998). *Bacillus subtilis*, in particular, had interaction in the rhizosphere with or without the presence of AMF. Synergistic interactions have been found between AMF and other beneficial soil microorganisms such as nitrogen-fixers (Sreenivasa and Bagyaraj, 1989; Biró et al., 2000).

Changes in bacterial community structure were shown to be driven by complex interactions between plant species (or genotype) and fungal species involved (Marschner and Baumann, 2003; Marschner and Timonen, 2005). However, bacterial communities in the mycorrhizosphere in turn influence the mycorrhizal or plant development in various ways, further complicating our understanding of bacterial-mycorrhizal interactions (Toljander, 2007). Due to the complex nature of the mycorrhizosphere, it has been difficult to trace the effects to specific factors/organisms. For example, the changes in microbial communities can be due to direct (e.g. belowground carbon allocation) or indirect influences of mycorrhizal colonisation (e.g. root exudation) or the combined effects of the mycorrhizal fungi and their biotic and abiotic environments (Toljander, 2007).

Conclusion

Rice production in Nigeria has encountered significant challenges, due to pests, diseases and soil fertility. A number of rice varieties that can withstand rice diseases have been developed and deployed in various places but the cost of fertilisation and the environmental impact of inorganic fertiliser have been one of the major setbacks in widespread productivity. This study investigated the deployment of six varieties of rice in the field with and without the inoculation of arbuscular mychorrizal fungi (AMF) consortium to determine if it can sustain soil fertility for rice production in two planting seasons and the corresponding influence

on soil microbial richness. The application of AMF not only increased important soil properties (pH, organic matter, total NPK) for plant growth, but it remarkably increased soil microbial population and activity. In addition, AMF inoculation increased plant yield and development parameters for optimum rice growth during both seasons. This study, however, requires further tests in other soils for extensive deployment.

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UTICAJI INOKULACIJE ARBUSKULARNO MIKORIZNIH GLJIVA NA OSOBINE ZEMLJIŠTA I PRINOS ODABRANIH VARIJETETA PIRINČA

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R e z i m e

Rast biljke može se stimulisati simbiotskom vezom između arbuskalarno mikoriznih gljiva (engl. *arbuscular mycorrhizal fungi* – AMF) i bakterija unutar regije rizosfere. Ove interakcije su ključne za povećanje plodnosti zemljišta, što vodi ka povećanoj produktivnosti i održivosti, kao i do prehrambene sigurnosti uzimajući u obzir visok nivo neuhranjenosti. Šest sorti pirinča uzgajane su sa (M+) ili bez (M-) inokulacije arbuskalarno mikoriznih gljiva po metodi slučajnog blok sistema sa tri ponavljanja. Fizičko-hemijske osobine zemljišta određene su uz pomoć standardnih metoda. Bakterije su izolovane iz uzoraka zemljišta i broj kolonija je određen tokom rane i kasne vegetativne sezone pirinča. Specifične osobine zemljišta (fosfat, pH, organska materija) znatno su se povećale u prisustvu arbuskalarno mikoriznih gljiva, što je vodilo do značajno većeg prinosa pirinča u obe berbe. Izolovane vrste bakterija obuhvatale su *Lactobacillus* spp., *Klebsiella aerogenes, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens*, *Azospirillum brasilense, Bacillus subtilis, Staphylococcus aureus, Enterobacter cloacae* i *Micrococcus* sp. Eksudati pirinča su povećali populaciju bakterija u ranoj žetvi, dok je tretman arbuskalarnim mikoriznim gljivama povećao populaciju bakterija kod kasne žetve i generalno povećao brojnost bakterijskih vrsta u obe sezone. Iako stvarni mehanizam koji je povećao brojnost bakterijskih vrsta nije bio poznat, ovim istraživanjem, međutim, pokazuje se da je interakcija arbuskalarno mikoriznih gljiva i bakterija povećavala i održavala plodnost zemljišta, što je zatim povećalo prinos pirinča. Dalja istraživanja su neophodna kako bi se odredio mehanizam interakcije koji je uočen između inokulacije arbuskalarno mikoriznih gljiva i populacije bakterija.

Ključne reči: mikoriza, fenolna jedinjenja, NERICA, kolonizacija.

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RESPONSE OF GROWING RABBITS (*ORYCTOLAGUS CUNICULUS*) FED DIETS CONTAINING RAW TALLOW (*DETARIUM MICROCARPUM*) SEED MEAL

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Abstract: A 12-week study was conducted to determine the effect of graded levels of raw tallow seed meal (RTSM) on growth performance, haematological and biochemical parameters and organoleptic qualities of growing rabbits. Five experimental diets were compounded to contain 0, 25, 50, 75 and 100% RTSM replacing the palm kernel cake weight for the weight designated as T1, T2, T3, T4 and T5, respectively. Forty-five (45) weaned rabbits between 5 and 6 weeks with an average body weight ranging from 500 to 600 g of mixed breeds and sexes (females and males) were randomly allocated to the five (5) dietary treatments in the randomized complete block design with nine (9) rabbits per treatment replicated three (3) times with three (3) rabbits each. Data were collected on feed intake, weight gain, feed conversion ratio, nutrient digestibility, some haematological and biochemical parameters and organoleptic qualities of the rabbits. The feed intake, weight gain and feed conversion ratio were significantly ($P<.005$) affected by the dietary treatments. The rabbits fed diets T1, T2 and T3 recorded similar feed intake (62, 66.95, and 66.50 g), total weight gain (1328.73, 1320.44, and 1323.49 g) and feed conversion ratio (3.92, 4.26, and 4.22) which were significantly better than those observed for the rabbits fed diets T4 and T5. The nutrient digestibilities of the rabbits fed the experimental diets were also significantly ($P<0.05$) affected. The rabbits fed diet T2 had better fiber digestibility (47.05%) compared to other treatment groups. Ether extract digestibility was observed to be better in the group of rabbits fed diets T1, T2 and T3, respectively. Some of the other nutrients in the group of rabbits fed diets T3, T4 and T5 were similarly digested. The haematological parameters were observed to be depressed as the level of RTSM increased in the diets. Packed cell volume, red blood cells and white blood cells were observed to reduce from 37.69 to 21.22%, 4.14 to 2.18

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g/dl and 4.98 to 3.02 g/dl. The biochemical parameters indicated a similar trend as that of the haematological parameters. Total protein, glucose and urea reduced from 6.15 to 4.63 g/dl, 5.15 to 3.80 g/dl and 7.76 to 4.00 mmol/l. The result of the organoleptic qualities indicated a non-significant (P>0.05) difference except for the juiciness which was significantly (P<0.05) high in the rabbits fed diets T1 (5.90), T3 (5.65), and T4 (5.90), respectively. In conclusion, up to 50% of RTSM can be included in the diets of rabbits without adverse effects on productive performance, nutrient utilization, blood parameters and organoleptic qualities.

Key words: rabbits, raw, tallow, seed meal, performance, nutrient digestibility.

Introduction

In Nigeria, there is an insufficient supply of cereals, cereal by-products and other agro-industrial wastes to sustain small- and medium-scale rabbit production (Makinde et al., 2017).To make rabbit rearing more viable as a small-scale business, Makinde and Inuwa (2015) advocated the utilization of cheap and locally available feedstuffs with a high nutritive value and digestibility.

One of such cheap and locally available ingredient is tallow(*Detarium microcarpum*)seeds. Tallow seed was reported by Uchegbu et al. (2009) to contain a sufficient amount of protein (17−36%), carbohydrate (39−66%) and fats (10−17%) with relatively adequate proportions of lysine and methionine as a percentage of the protein (2.14 and 1.0% respectively). In the feeding trial conducted by Obun et al. (2011), the authors reported thatwhen tallow seed meal was boiled for 80 minutes, about 20% was incorporated in the diet of starter chicks without any adverse effects on growth performance. Furthermore, Obun (2013) reported that raw tallow seed meal can be included in the broiler diet up to the 5% level without influencing the acceptability of the feed and haematological and biochemical indices of broilers.However, there is a dearth of information on the potential of *D. microcarpum*seed meal as an alternative feed source for rabbits. It was in view of the above that this study was conducted to investigate productive performance, blood constituents, nutrient digestibility and organoleptic properties of rabbits fed raw tallow seed meal based diets.

Materials and Methods

Experimental site

The study was carried out at the Rabbitry Unit of the Ministry of Livestock Services and Development, State Veterinary Centre, Bosso, Minna, Niger State. Minna is located on the latitude 9^0N, longitude 7^0E. Minna experiences distinct dry

and wet seasons with its mean annual rainfall of 1100 and 1600 mm (with the highest main monthly rainfall in September) and a mean temperature ranges between 21^0C and 36.5^0C (Climatemp, 2011).

Source of feed ingredients and experimental diets

Tallow seeds were sourced from Bida and its surrounding villages, while the fixed ingredients such as premix, bone meal, oyster shell, maize, PKC and maize offal were obtained from Sammy Agro-ventures Milling Center, Minna, Niger State while the common salt was obtained from Kure Ultra-modern Market, Minna. Raw tallow seed (50 kg) was crushed using a crushing machine before taken to Sammy Agro-venture milling center to formulate the rabbit diets. Five dietary treatments were formulated. Diet T1 (control diet) contained 0% raw tallow seed meal, while T2, T3, T4 and T5 contained 25, 50, 75 and 100% raw tallow seed meal respectively with the tallow seed being quantitatively substituted for palm kernel cake (PKC). The proximate composition of raw tallow seed is shown in Table 1 while the gross composition of experimental diets is shown in Table 2.

Table 1. Proximate composition of raw tallow seed.

Parameters	Composition (%)
Dry matter	92.00
Crude fiber	14.95
Crude protein	19.25
Ether extract	8.38
Ash	3.50
Nitrogen-free extract	46.62
Energy (kcal/kg ME)	3300.60

NFE = 100 – (% moisture + % crude protein + % ether extract + % crude fiber + % ash).

Experimental rabbits and their management

The experiment was conducted using forty-five (45) weaned rabbits of different breeds and mixed sexes. The rabbits were purchased from the Rabbitry Unit of the Ministry of Livestock Service and Development, State Veterinary Center Bosso, Minna, Niger State, Nigeria. The rabbits were between 5 and 6 weeks of age with an average initial weight ranging between 500 and 600 g. Before arrival of the rabbits, hutches were washed, cleaned and disinfected using detol and detergent. Also, both the feeders and drinkers were washed and cleaned. The hutches, feeders and drinkers were cleaned and dried on a daily basis before water and feeds were supplied as prescribed by Aduku and Olukosi (1990).

There was an adaptation period of 4–5 days for the animals to become accustomed to the environment and new feed. The rabbits were randomly allocated

to five treatments in a completely randomized design. There were nine rabbits in each treatment with three rabbits per replicate. The routine veterinary care was given throughout the period of the experiment. All the rabbits were dewormed against ecto and endo parasites using Ivermectin injection.

Table 2. The gross composition of the experimental diets.

Ingredient (%)	$T_{1\ (0\%)}$	$T_{2\ (25\%)}$	$T_{3\ (50\%)}$	$T_{4\ (75\%)}$	$T_{5\ (100\%)}$
Maize	17.05	17.05	17.05	17.05	17.05
PKC	58.10	43.57	29.05	14.53	0.00
RTSM	0.00	14.53	29.05	43.57	58.10
Maize offal	20.00	20.00	20.00	20.00	20.00
Salt	0.50	0.50	0.50	0.50	0.50
*Vitamin premix	0.25	0.25	0.25	0.25	0.25
Bone meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	1.10	1.10	1.10	1.10	1.10
Total	100.00	100.00	100.00	100.00	100.00
Determined nutrients (%)					
Crude protein	16.85	17.00	17.25	16.45	17.00
Crude fiber	15.62	16.28	15.11	14.23	14.68
Ash	8.50	10.00	12.50	13.50	10.50
Ether extract	9.00	8.50	10.50	9.50	9.50
Energy,Kcal/kg ME	2523.00	2620.00	2632.00	2721.00	2641.00

T_1(0% RTSM), T_2(25% RTSM), T_3(50% RTSM), T_4(75% RTSM), T_5(100% RTSM) *premix supplied contains: retinol acetate (1000000 iu), Vit D3, (20000000 iu), Vit E (1500 iu), Vit B (3000 mg), Niacin (1500 mg), Calcium pantoethenate (800 mg), Vit B6 (300 mg), Vit B12 (10 mg), Vit K3 (2000 mg), Biotin (20 gm), Folic acid (500 mg), Choline chloride (250000 mg), Manganese (75000 mg),Iron (2500 mg), Copper (5000 mg), Zinc (7000 mg), Selenium (150 mg), Iodine (1300 mg), Magnesium (100 mg), 500g ethoxyquin and BHT (700 g).

Data collection

Feed consumption was calculated on a daily basis using the formula:

Feed consumption = amount of feed given (g) – amount of left-over feed (g).

The animals were weighed individually on a weekly basis to determine the weight changes, and the weight gained was determined by the formula shown below:

$$Dailyweightgained/rabbits/day = \frac{finalweight(g) - initialweight(g)}{numberofrabbits \times numberofdays}.$$

Feed conversion ratio (FCR) was also calculated as the quantity of feed that would produce 1 kg of weight gain using the formula:

$$Feedconversionratio(FRC) = \frac{totalfeedintake}{totalweightgain}.$$

Digestibility trial

The digestibility test was carried out using the complete collection method at the eleventh (11) week of the study. The rabbits were placed in their respective metabolic cages according to their treatment and were adapted for three (3) days. Faecal samples were collected for five days, air-dried, bulked together and maintained with boric acid independently in a dark plastic bag and then saved in the fridge in the laboratory with the number label on each nylon, for easy identification. The faecal samples were oven-dried and the differences were taken between the wet and dried faecal samples and the sub-samples were analyzed for proximate composition. The percentage of nutrient retention was calculated using the equation below:

Nutrient retention = [(nutrient intake-nutrient in excreta)/(nutrient intake)] × 100,

where:

Nutrient intake (g) = dry feed intake × nutrient in diet,

Nutrient in excreta (g) = dry faecal output × nutrient in faeces.

Haematological and blood serum analysis

At the end of the trial, two rabbits per replicate were randomly selected for blood analysis at the 12^{th} week. They were bled from the ear vein. About 5 ml of blood for haematological analysis was collected from each rabbit into bottles containing ethylene diamine tetra acetic acid (EDTA). Blood samples meant for determining biochemical indices (total protein, albumin, globulin, urea, cholesterol and triglyceride) were collected into the bottles without EDTA. Haematological and biochemical parameters were determined using the standard clinical chemistry procedure (Olorede et al., 1996).

Sensory evaluation

The meat from the hind limbs was boiled and used for the sensory evaluation. Various cuts of the meat were made into bite sizes and then served in coded plates to twenty (20) qualified panel members to evaluate the meat for color, bitterness, juiciness, taste and overall acceptability using the 9-point hedonic scale (1 – hate incredibly, 9 – like extremely). The order of demonstration of samples was randomized to the panelists. Each of these palatability characteristics was ranked independently of others. Cool water was provided to the judges to rinse their mouths after scoring each sample.

Statistical analysis

All data collected were subjected to one-way analysis of variance (ANOVA). Differences between means were separated using Duncan's multiple range test (Duncan, 1955). All computations were made by statistical software (SPSS, 2006).

Results and Discussion

The results of growth performance of rabbits fed raw tallow seed meal (RTSM) are presented in Table 3. The feed intake, weight gain and feed conversion ratio (FCR) were significantly (P<0.05) affected. Similar weight gains and feed conversion ratio were observed in the groups of rabbits fed diets T1, T2 and T3, respectively. The lowest feed intake, weight gain and poor feed conversion ratio were recorded in the groups of rabbits fed diets T4 and T5. However, the low feed intake was similarly recorded in the group of rabbits fed diet T1. The decrease in the final live weight, feed intake, weight gain and poor FCR observed among rabbits fed diets T4 and T5 is in line with a similar observation made by Obun et al. (2011), for broiler chickens fed raw tallow diets. This could be attributed to the inherent anti-nutritional factors present in the raw tallow seed meal (RTSM) such as oxalates, phytates, saponins and tannins. Oxalates form complexes with the mineral such as calcium and thus make them unavailable to the body, resulting in low feed intake and causing irritation of the gut, inhibiting energy and protein utilization in broilers (Agwunobi et al., 2002; Okereke, 2012). Phytates affect protein and mineral utilization, which results in the poor performance while tannins impair digestive enzymes and cause gut irritation. Also, oxalates impair the absorption of calcium in the digestive tract and limit nitrogen retention (Hang and Binh, 2013). Olomu (1995) has reported that saponin impairs the performance through its irritating effect on the linings of the mouth and guts and through its bitter taste. Tannin in diets imposes an astringent taste that affects palatability, reduces feed consumption and consequently growth performance. It also binds to both endogenous and exogenous proteins including enzymes of the digestive tract, thereby affecting the protein utilization (Sotelo et al., 1995). The improved performance observed among rabbits fed 25 and 50% RTSM diets in terms of body weight gain, feed intake and feed conversion ratio suggests that there was an enhanced availability, digestion, absorption and utilization of the nutrients by the rabbits.

Table 3. Growth performance of rabbits fed graded levels of raw tallow seed meal.

Parameters	$T_{1\ (0\ \%)}$	$T_{2\ (25\ \%)}$	$T_{3\ (50\ \%)}$	$T_{4\ (75\ \%)}$	$T_{5\ (100\ \%)}$	SEM	LS
Average initial weight, g/r	505.44	504.45	506.40	506.11	506.11	0.98	NS
Average final weight, g/r	1834.17^{a}	1824.89^{a}	1829.89^{a}	1637.00^{b}	1626.00^{b}	28.00	*
Total weight gain, g/r	1328.73^{a}	1320.44^{a}	1323.49^{a}	1130.89^{b}	1119.89^{b}	26.15	*
Average daily weight gain, g/r	15.82^{a}	15.72^{a}	15.76^{a}	13.46^{b}	13.33^{b}	0.28	*
Total feed intake, g/r	5208.00^{b}	5623.80^{a}	5586.00^{a}	5812.80^{a}	5376.00^{ab}	134.40	*
Average feed intake, g/r	62.00^{b}	66.95^{a}	66.50^{a}	69.20^{a}	64.00^{ab}	1.60	*
FCR	3.92^{a}	4.26^{a}	4.22^{a}	5.14^{b}	4.80^{b}	0.15	*
Mortality, %	0.00	0.00	0.00	0.00	0.00		

^{abc}means with different superscripts in the same row are significantly (p<0.05) different. SEM = standard error of the mean, LS = level of significance, g/r = grams per rabbit, FCR = feed conversion ratio.

The results of nutrient digestibility of rabbits fed RTSM based diet are shown in Table 4. The results show that digestibilities for crude protein, crude fiber, ether extract and nitrogen-free extract were significantly ($p<0.05$) different while dry matter showed no significant ($p>0.05$) difference in all treatments. Crude protein digestibility of the rabbits fed T1 diet was significantly ($p<0.05$) higher than those of the rabbits fed other diets. The crude fiber digestibility showed that rabbits fed diets T1 and T2 had significantly ($p<0.05$) higher values than those fed diet T5 which had the lowest value (39.49%). Ether extract digestibility was similar ($p>0.05$) for rabbits fed diets T1, T2 and T3. The lowest values were recorded among rabbits fed T4 and T5 diets ($p>0.05$). Nitrogen-free extract digestibility was significantly ($p<0.05$) higher among rabbits fed diets T1, T2 and T3 than among those fed other diets. The results of the digestibility studies show that there was a significant ($P<0.05$) reduction in the digestibility of nutrients with increasing levels of RTSM in the diets. Rabbits fed diets T1, T2 and T3 had similar ether extract and nitrogen-free extract digestibilities which could be an indication that the rabbits could tolerate RTSM up to the 50% inclusion level in the diets. The reduction in crude protein digestibility and other nutrients with increased RTSM may be ascribed to the presence of the anti-nutritional substances contained in the raw tallow seed meal. The anti-nutritional factors (ANFs) interfere with metabolic processes such as growth and bioavailability of nutrients that are negatively influenced (Binita and Khetarpaul, 1997). Like other ANFs, the intake of a sufficient quantity of dietary tannin resulted in decreased daily gain and impaired the efficiency of feed utilization due to reduced protein digestibility in chickens (Obun et al.,2011) and pigs (Jansman et al.,1993). Tannins have been reported to contain a high protein binding capacity, that is, tannin-protein complexes, which are extremely hydrophobic (Mitaru et al., 1984) and have been reported to be responsible for low digestibility of protein and low availability of amino acids due to increased faecal excretion of nitrogen in pigs (Hlodversson, 1987) and chickens (Ortiz et al., 1993).

Table 4. Nutrient digestibility of rabbits fed graded levels of raw tallow seed meal.

Parameters (%)	$T_{1\ (0\ \%)}$	$T_{2\ (25\ \%)}$	$T_{3\ (50\ \%)}$	$T_{4\ (75\ \%)}$	$T_{5\ (100\ \%)}$	SEM	LS
Dry matter	85.05	81.13	81.12	82.10	81.70	0.49	NS
Crude protein	84.19^{a}	79.83^{b}	80.85^{b}	80.64^{b}	79.97^{b}	0.62	*
Crude fiber	47.57^{a}	47.05^{a}	43.61^{b}	45.70^{b}	39.49^{c}	0.63	*
Ether extract	92.93^{a}	93.86^{a}	93.72^{a}	81.88^{b}	82.88^{b}	1.16	*
NFE	87.69^{a}	85.12^{a}	84.04^{a}	81.49^{c}	78.89^{d}	1.74	*

^{abc}means with the same superscript in the same row are significantly different ($p<0.05$). LS = level of significance, SEM = standard error of the mean, NFE = nitrogen-free extract.

The results of haematological parameters of rabbits fed RTSM based diets are shown in Table 5. The results reveal that packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), mean corpuscular volume (MCV), neutrophils, lymphocyte, and basophil were significantly ($p<0.05$) affected by the dietary treatments. The lower values for white blood cell, lymphocytes, neutrophils and basophil counts as observed among rabbits fed diets T3, T4 and T5 could be attributed to the accumulative toxic effect of high RTSM levels in the diets. This is similar to the report of Obun (2013), who observed a decrease in the white blood cell as raw tallow seed meal increased in the diet of broiler chickens. The non-significant effect of the diet on red blood cells was an indication that the diet had no influence on the red blood cells. Fanimo et al. (2003) had earlier reported that the inclusion of cashew apple waste in the diet of growing rabbits did not affect the red blood cell. The values of haemoglobin of rabbits fed diets T4 and T5 (9.25 and 8.10 g/dl) fell below the normal range of 10–15 g/dl and the PCV values of rabbits fed diets T2, T3, T4 and T5 (27.18, 28.55, 13.94 and 21.22%) also fell below the normal range of 30–50% reported by RAR (2009). Lloyd and Gibson (2006) observed that normal haemoglobin and PCV values are good indicators of the nutritional status of the feed. Mean corpuscular volume was significantly higher among rabbits fed diet T3 compared with those fed other diets.

Table 5. Haematological values of rabbits fed graded levels of raw tallow seed meal.

Parameters	$T_{1\ (0\ \%)}$	$T_{2\ (25\ \%)}$	$T_{3\ (50\ \%)}$	$T_{4\ (75\ \%)}$	$T_{5\ (100\ \%)}$	SEM	LS
PCV (%)	37.69^{a}	27.18^{b}	28.55^{b}	13.94^{c}	21.22^{b}	2.77	*
Hb (g/dl)	11.35^{a}	11.50^{a}	11.60^{a}	9.25^{b}	8.10^{b}	0.94	*
RBC ($\times10^{6}$/mm^{3})	4.98	4.05	3.00	3.38	3.02	0.37	NS
WBC ($\times10^{3}$/mm^{3})	6.29^{a}	6.14^{a}	4.15^{ab}	3.30^{bc}	2.18^{c}	0.70	*
MCH (pg)	19.90	19.70	19.85	17.00	18.70	0.63	NS
MCV (fl)	67.50^{b}	76.50^{b}	91.50^{a}	77.00^{b}	76.00^{b}	2.84	*
MCHC (g/dl)	21.25	28.65	28.15	23.85	28.35	1.26	NS
Neutrophils (%)	69.38^{a}	60.27^{b}	58.25^{b}	58.85^{b}	49.95^{c}	2.59	*
Lymphocyte (%)	28.30^{a}	30.10^{a}	23.65^{ab}	17.20^{b}	13.15^{b}	2.56	*
Monocyte (%)	5.58	5.00	13.45	11.40	17.40	1.83	NS
Basophil (%)	3.53^{a}	2.95^{a}	0.20^{b}	0.20^{b}	0.20^{b}	0.53	*

^{abc}means with different superscripts in the same column are significantly ($p<0.05$) different. SEM = standard error of the mean, NS = not significant, *significant, PCV = packed cell volume, Hb = haemoglobin, RBC = red blood cell, WBC = white blood cell, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume.

The results of serum biochemical indices of rabbits fed RTSM based diets are shown in Table 6. The results show that total protein, albumin, cholesterol, triglyceride and urea were significantly ($p<0.05$) affected by the dietary treatments. The observed decrease in total protein, albumin and urea concentrations among rabbits fed diets T4 and T5 suggests alteration of normal protein metabolism due to

interference of protein utilization. This result is in line with similar observation made by Obun (2013), for broiler chickens fed raw tallow diets. The decrease in serum cholesterol among rabbits fed diet T4 may be attributed to the metabolites present in RTSM. Price et al.(1987) had earlier reported that saponin in diets of animals is known to reduce the uptake of certain nutrients such as cholesterol and glucose, and may help in reducing the metabolic burden that would have been placed on the liver. Similarly, Awe and Sodipo (2001) observed that saponin reduces body cholesterol by preventing the bile reabsorption and suppresses rumen protozoan thereby causing it to lyse. Triglyceride was significantly higher among rabbits fed diets T2 and T3, but differed ($p<0.05$) significantly from all other treatment means which were similar ($p>0.05$).

Table 6. Serum biochemical indices of rabbits fed graded levels of raw tallow seed meal.

Parameters	$T_{1\ (0\ \%)}$	$T_{2\ (25\ \%)}$	$T_{3\ (50\ \%)}$	$T_{4\ (75\ \%)}$	$T_{5(100\ \%)}$	SEM	LS
Total protein (g/dl)	6.15^{a}	5.70^{ab}	5.90^{a}	4.95^{b}	4.63^{b}	0.23	*
Albumin (g/dl)	4.10^{a}	3.65^{ab}	3.80^{a}	2.80^{b}	2.45^{b}	0.31	*
Globulin (g/dl)	2.05	2.05	2.10	2.15	2.18	0.18	NS
Cholesterol (g/dl)	1.25^{ab}	1.85^{a}	1.85^{a}	0.60^{b}	1.25^{ab}	0.17	*
Triglyceride (g/dl)	0.30^{b}	1.75^{a}	1.55^{a}	0.60^{b}	0.70^{b}	0.19	*
Glucose (g/dl)	5.15	3.85	4.35	4.75	3.80	0.23	NS
Urea (mmol/L)	7.76^{a}	6.66^{ab}	7.75^{a}	4.26^{b}	4.00^{b}	0.61	*

^{a,b,c}means with different superscripts in the same row are significantly ($p<0.05$) different. SEM = standard error of the mean, LS = level of significance.

The results of the sensory evaluation of meat from rabbits fed RTSM based diet are shown in Table 7. The results reveal that tenderness, flavor, color and overall acceptability were not significantly ($p>0.05$) different except for the juiciness. The non-significant difference ($p>0.05$) observed in this study on the organoleptic qualities of boiled meat from rabbits fed graded levels of RTSM is in line with the report of Vasanthakumar et al.(1999) that sensory attributes of pressure-cooked meat from weaned rabbits fed graded levels of neem seed kernel cake were found to be similar.

Table 7. Sensory evaluation of meat from rabbits fed graded levels of raw tallow seed meal.

Parameters	$T_{1\ (0\ \%)}$	$T_{2\ (25\ \%)}$	$T_{3\ (50\ \%)}$	$T_{4\ (75\ \%)}$	$T_{5\ (100\ \%)}$	SEM	LS
Tenderness	5.25	5.10	5.05	4.55	5.00	0.13	NS
Flavor	5.85	6.80	6.20	6.10	6.05	0.20	NS
Juiciness	5.90^{a}	5.00^{b}	5.65^{ab}	5.90^{a}	4.60^{b}	0.19	*
Color	5.90	5.75	5.50	5.60	5.90	0.20	NS
Overall acceptability	6.70	7.15	6.75	5.95	6.20	0.41	NS

^{abc} means with the same superscript in the same column are significantly ($p<0.05$) different. LS = level of significance, SEM = standard error of the mean.

Conclusion

This study shows that up to 50% of RTSM can be included in the diets of rabbits without adverse effect on productive performance, nutrient utilization, blood parameters and organoleptic qualities.

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ODGOVOR UZGAJANIH KUNIĆA (*ORYCTOLAGUS CUNICULUS*) HRANJENIH SAČMOM SIROVOG SEMENA BILJKE *DETARIUM MICROCARPUM*

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R e z i m e

Dvanaestonedeljno istraživanje sprovedeno je kako bi se utvrdio uticaj unosa različito graduisane sačme sirovog semena biljke *Detarium microcarpum* (engl. *raw tallow seed meal* – RTSM) na učinak rasta, hematološke i biohemijske parametre i organoleptičke osobine uzgajanih kunića. Pet eksperimentalnih obroka sastavljeni su tako da sadrže 0, 25, 50, 75 i 100% RTSM zamenjujući težinu pogače palminog zrna za težinu označenu kao T1, T2, T3, T4 odnosno T5. Četvrdeset pet (45) odbijenih kunića mešovitih rasa i polova (muškog i ženskog) starosti između 5 i 6 meseci sa prosečnom telesnom težinom od 500 do 600 g nasumično su podeljeni u pet (5) dijetetskih tretmana po metodu slučajnog potpunog blok dizajna sa devet (9) kunića po tretmanu koji je ponovljen tri (3) puta sa tri (3) kunića po ponavljanju. Prikupljeni su podaci o uzimanju hrane, prirastu težine, odnosu utroška hraniva prema jedinici prirasta, svarljivosti hranljivih materija, nekim hematološkim i biohemijskim parametrima i organoleptičkim osobinama kunića. Uzimanje hrane, prirast težine i odnos utroška hraniva prema jedinici prirasta bili su značajno (P<,005) uslovljeni dijetetskim tretmanima. Kod kunića koji su hranjeni obrocima T1, T2 i T3 zabeleženo je slično unošenje hrane (62, 66,95, i 66,50 g), ukupan prirast težine (1328,73, 1320,44, i 1323,49 g) i odnos utroška hraniva prema jedinici prirasta (3,92, 4,26, i 4,22) koji su bili značajno bolji nego vrednosti zabeležene kod kunića hranjenih obrocima T4 i T5. Svarljivost hranljivih materija kunića hranjenih ekperimentalnim obrocima takođe je bilo značajno (P<0,05) uslovljeno. Kunići hranjeni obrokom T2 imali su bolju svarljivost vlakana (47,05%) u poređenju sa drugim grupama. Zabeleženo je da je svarljivost ekstrakta etra bolja u grupi kunića hranjenih obrocima T1, T2 odnosno T3. Neke druge hranljive materije u grupi kunića hranjenih obrocima T3, T4 i T5 bile su slično svarene. Uočeno je da su se hematološki parametri smanjivali kako se nivo RTSM povećavao u obrocima. Primećeno je da se hematokrit, crvena krvna zrnca i bela krvna zrnca smanjuju sa 37,69 na 21,22%, sa 4,14 na 2,18 g/dl i sa 4,98 na 3,02 g/dl. Biohemijski parametri ukazuju na sličan trend koji postoji i kod

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hematoloških parametara. Ukupni proteini, glukoza i urea se smanjuju sa 6,15 na 4,63 g/dl, sa 5,15 na 3,80 g/dl i sa 7,76 na 4,00 mmol/l. Rezultat organopetičkih osobina pokazuje manje značajnu (P>0,05) razliku osim za sočnost koja je značajno (P<0,05) visoka kod kunića hranjenih obrocima T1 (5,90), T3 (5,65), odnosno T4 (5,90). Da zaključimo, do 50% RTSM može se uključiti u obroke kunića bez štetnih uticaja na produktivni učinak, iskorišćenost hranljivih materija, hematološke parametre i organoleptičke osobine.

Ključne reči: kunići, sirov, *Detarium microcarpum*, sačma, učinak, svarljivost hranljivih materija.

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„VODNI OTISAK" U POLJOPRIVREDI I VIRTUELNA TRGOVINA VODOM. DA LI SRBIJA IZVOZI ILI UVOZI VODU?

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Sažetak: Ograničeni vodni resursi, rastući zahtevi za vodom i sve nepovoljniji klimatski uslovi doveli su do razvoja novih koncepata, sa ciljem procene potražnje i potrošnje vode na lokalnom i globalnom nivou. Koncepti koji se u novije vreme koriste pri rešavanju ovakvih problema su „vodni otisak" i „virtuelna trgovina vodom". Ciljevi ovog rada su: (1) da se odrede specifični zahtevi za vodom najvažnijih poljoprivrednih proizvoda u procesu međunarodne trgovine Srbije, (2) da se oceni mogućnost povećanja produktivnosti vode pri proizvodnji tih proizvoda i (3) da se proceni održivost vodnih resursa Srbije, na osnovu odnosa vode koju Srbija uvozi/izvozi tokom „virtuelne trgovine vodom". Izračunavanjem specifične potrošnje vode za pšenicu, kukuruz, suncokret, šećernu repu i soju i poređenjem sa specifičnom potrošnjom vode ovih kultura u drugim zemljama, utvrđeno je da postoji prostor da se unapredi korišćenje vode, npr. podešavanjem sortimenta ili promenama u tehnologiji gajenja. Na osnovu odnosa izračunatih izvezenih i uvezenih virtuelnih količina vode za period 1995–1999. godine i 2010–2013. godine, može se zaključiti Srbija izvozi više vode nego što uvozi. Čak i sa dodatnim porastom izvoza poljoprivrednih proizvoda neće doći do narušavanja vodne održivosti Srbije.

Ključne reči: specifična potrošnja vode, virtuelna trgovina vodom, poljoprivredni proizvodi, produktivnost vode.

Uvod

Poljoprivreda je najveći potrošač vode na svetu sa prosečnom potrošnjom od oko 70% ukupnih voda (FAO, 2017). Ovaj procenat je u aridnim predelima znatno veći i ide do 89%, dok je u humidnim predelima značajno manji i varira od 20% u Rusiji, do 35–60% u tropskim i humidnim predelima (FAO, 2017). Sa porastom

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broja stanovnika i potrebe za hranom, uz klimatske promene, potražnja za vodom će biti sve veća. U uslovima ograničenih vodnih resursa i nepovoljnih klimatskih promena koje narušavaju bilans voda, realna je opasnost konflikata, od lokalnih i nacionalnih, preko regionalnih do globalnih.

Da bi se procenila potražnja i potrošnja vode u različitim zemljama, Allan (1993) je razvio koncept „virtuelne trgovine vodom" (VTV) i predložio dva pristupa: prvi, da se odredi zapremina vode putem tzv. „vodnog otiska" (engl. *Water Footprint Network*) (Hoekstra i Hung, 2002; Hoekstra i Chapagin, 2006; Hoekstra et al., 2011), i drugi, da se analizira potrošnja vode od početka do kraja proizvodnog procesa (engl. *life-cycle analysis*) (Hoekstra et al., 2011). Pojam „virtuelna voda" podrazumeva količinu vode koja se koristi u procesu proizvodnje poljoprivrednog ili industrijskog proizvoda (Hoekstra i Hung, 2002), a koji je predmet međunarodne trgovine. Dakle, treba izračunati koliki je specifični zahtev jednog proizvoda za vodom (SZV) izražena u kubnim metrima po toni ($m^3 t^{-1}$), što je u stvari recipročna vrednost vodne produktivnosti obično izražene u kilogramima po kubnom metru (vode) ($kg\ m^{-3}$).

Na globalnom nivou, ušteda vode se ostvaruje ako se proizvod izvozi iz zemlje gde je vodna produktivnost veća u zemlju gde je produktivnost manja. Pod uštedom se obično podrazumeva količina vode koja bi se potrošila da je proizvod proizveden lokalno; količine uštеđene vode variraju značajno od zemlje do zemlje (Yang et al., 2006). Znajući koliki su vodni resursi jedne zemlje i koliko je vode potrebno za proizvodnju svakog pojedinog proizvoda, moguće je napraviti strategije razvoja privrede i onih proizvoda koji u svom procesu koriste manje vode, tako da se voda efikasnije koristi, a da se pri tome obezbedi održivost obnovljivih vodnih resursa (Aldaya et al., 2010). U Maroku je urađena studija sa ciljem da se utvrde virtuelni tokovi vode koji ulaze i izlaze iz zemlje, radi formulisanja nacionalne politike vode (Schyns i Hoekstra, 2014). Oni su došli do zaključka da se do uštede vode može doći ukoliko se pojedini usevi gaje na slivovima gde su zahtevi poljoprivrednih kultura manji, u odnosu na postojeću reonizaciju i da se smanji vodni otisak useva do standardnih uspešnih nivoa. U sušnim zemljama, sa nedovoljnim vodnim resursima za potrebe poljoprivrede, poput Tunisa, ušteda vode može da se ostvari tako što bi se uvozile one osnovne životne namirnice za koje je neophodno utrošiti mnogo vode umesto da se gaje u domicilnim zemljama (Chouchane et al., 2018).

U stvari, treba težiti da se unapredi vodna produktivnost svakog proizvoda. Mnoge kompanije su već ozbiljno shvatile problem nestašice vode, pristupile su analizi proizvodnih procesa i prilagodile ih tako da se voda koristi na efikasan način. To je rezultiralo da se potrošnja sveže vode drastično smanji u nekim kompanijama i do 27% (Zhang et al., 2013).

Cilj ovog rada jeste da se ustanove specifični zahtevi za vodom (SZV) najvažnijih proizvoda u procesu međunarodne trgovine poljoprivrednim

proizvodima, da se uporede SZV sa rezultatima nekoliko izabranih zemalja koje su ustanovili Chapagin et al. (2004), da se utvrdi da li je moguće povećati produktivnost vode, i da se utvrdi da li Srbija više izvozi ili uvozi vode i da li se obezbeđuje održivost vodnih resursa.

Materijal i metode

Da bi se izračunala količina virtuelne vode nekog proizvoda, neophodno je da se odvojeno računa za proizvode biljnog i životinjskog porekla, a posebno za proizvode industrijskog porekla. Detaljna analiza izračunavanja virtuelne vode za proizvode biljnog porekla, poput pšenice, kukuruza, soje, suncokreta i drugih najvažnijih ratarskih, voćarskih i povrtarskih kultura prikazana je u publikaciji Hoekstra i Hung (2002).

Da bi se izračunala virtuelna voda, neophodno je odrediti potrebu svakog pojedinačnog useva za vodom (PV) u m^3 ha^{-1} za određeni region ili celu državu, na osnovu eksperimentalnih istraživanja ili izračunavanjem pomoću modela. Za ovu svrhu se najčešće koristi FAO model CROPWAT (www.fao.org). Potrebni ulazni podaci za ovaj model jesu:

- klimatski podaci o srednjim mesečnim minimalnim i maksimalnim temperaturama vazduha, relativnoj vlažnosti vazduha, brzini vetra, insolaciji i padavinama;
- podaci o kapacitetima zemljišta za vodu;
- podaci o kulturama (datumi setve, trajanje fenofaza, datumi žetve, osetljivost na vodni stres, koeficijenti kulture po fazama potrošnje).

Zatim je potrebno raspolagati prinosima po regionima ili za celu zemlju u t ha^{-1}. Prinosi dobijeni eksperimentalnim istraživanjima obično se razlikuju od statističkih, tako da je za procenu obima virtuelne trgovine vodom realnije koristiti statističke prinose, dok se eksperimentalni mogu koristiti za poboljšanje efikasnosti korišćenja vode u strateškim planiranjima.

Na osnovu ove dve vrednosti moguće je izračunati specifični zahtev za vodom (SZV) nekog prinosa, odnosno koliko je kubnih metara vode potrebno da se dobije jedna tona biljnog proizvoda (m^3 t^{-1}).

Virtuelna trgovina vodom (VTV) predstavlja proizvod specifičnog zahteva za vodom (SZV) i ukupne količine nekog proizvoda koji se izvozi ili uvozi (P):

$$VTV = \sum_{u,i} SZV_{u,i} * P_{u,i}.$$

Hoekstra i Hung (2002) su izračunali SZV kultura koje su najčešće učestvovale u spoljnotrgovinskom prometu, po navedenoj proceduri na osnovu raspoloživih klimatskih podataka i statističkih prinosa za Jugoslaviju u periodu 1995–1999. godine (tabela 1).

Kao dopunu, ovde dajemo i vrednosti SZV izvedene na osnovu statističkih podataka o prinosima za Srbiju u periodu 1998–2012. godine preuzetih sa sajta

Republičkog zavoda za statistiku (www.stat.gov.rs). Podaci potrebni za izračunavanje potrošnje vode zasnovani su takođe i na osnovu eksperimentalnih istraživanja i obračuna potreba useva za vodom (tabela 1).

Tabela 1. Specifični zahtevi za vodom kultura koje su značajne u spoljno-trgovinskoj razmeni.
Table 1. Specific water demand of crops important in international trade.

Usev/*Crop*	Prinos* *Yield** (t/ha)*	Prosečan prinos *Average yield* (t/ha)	Potreba useva za vodom (PV)/*Crop water requirement* (CWR) (m^3/ha)	SZV *SWD* (m^3/t)	SZV^e SWD^e (m^3/t)	SZV^N SWD^N (m^3/t)
Kukuruz/*Maize*	11,9	4,5	4950	416	1098	1009
Pšenica/*Wheat*	7,3	3,5	5700	781	1619	1743
Šećerna repa/*Sugar beet*	80,5	40,8	6450	80	158	175
Ječam/*Barley*		2,6	4100			1599
Ovas/*Oat*		2,2	4160			2188
Proso/*Millet*		0,6	4130			6883
Raž/*Rye*		2,0	5000			2500
Suncokret/*Sunflower*	3,3	2,0	4800	1455	2426	3107
Uljana repica/*Oilseed rape*		2,0	5000	2500		
Soja/*Soya bean*	3,5	2,3	4500	1286	1924	1925
Lucerka/*Alfalfa*	17	5,218	6100	359	1169	37***
Paprika/*Pepper*	90	7,5	6500	72	873	3207
Sirak/*Sorghum*		4,7	4010			861
Paradajz/*Tomato*	170	8,6	5500	32	638	697
Krompir/*Potato*	42	9,7	4600	110	475	621
Kupus/*Cabbage*	59	13,8	4100	70	297	178
Grašak/*Peas*	10	2,4 (5,6)[H]	4000	400	1643	627
Crniluk/*Onion*	30	6,4 (20)[H]	4500	150	703	210
Krastavac/*Cucumber*		7,4	3620			491
Pasulj/*Dry bean*		1,3	4190			3136
Boranija/*Green bean*		4,2	3400			816
Povrće/*Vegetable*		7,6	3770			496
Lubenica/*Water melon*		13,6	4620			340
Duvan/*Tobacco*		1,7	4370			2646
Grožđe/*Grape*		3,6	4390			1237
Šljiva/*Plum*	50	32,8	6000	667	120	
Jabuka/*Apple*	80	41,5	5150	65	468	
Jagoda/*Strawberry*		4,0	5000	1250		
Kafa/*Coffee*						2697**

[N] Vrednosti preuzete od Hoekstra i Hung (2002); *ostvareni prinosi eksperimentalnim putem; ** prosečna vrednost za neprženu kafu; *** vrednost za stočnu hranu, SZV – specifični zahtevi kulture za vodom dobijeni na osnovu prosečnih prinosa, SZV^e– specifični zahtevi kulture za vodom izračunati na osnovu prinosa dobijenih eksperimentalnim putem.

*[N] – Source: Hoekstra and Hung (2002); * yield obtained by experimental trials; ** average value for raw coffee; *** value for forages; SWD – specific water demand based on average yield; SWD^e– specific water demand based on yields obtained in experimental trials.*

Iz publikacije Hoekstra i Hung (2002) preuzete su i vrednosti specifičnih zahteva za vodom kukuruza, šećerne repe, pšenice, soje i suncokreta iz konkurentnih zemalja da se utvrdi da li se u Srbiji efikasnije koristi voda i da li postoji mogućnost za unapređenje. Podaci o spoljno-trgovinskoj razmeni za period 2010–2013. godine mogu se preuzeti sa sajta FAO (http://www.fao.org/faostat/en/#data/TP), a za 2016. godinu iz baze podataka sa sajta Republičkog zavoda za statistiku.

S obzirom na to da je Srbija veliki izvoznik mleka, šećera, ulja i masti, neophodno je primeniti analizu virtuelne vode proizvoda životinjskog porekla. Ova procedura je složenija, jer uzima u obzir virtuelnu vodu hrane koju životinje konzumiraju tokom života, zatim koliko vode popiju (svinje, ovce, junad...) i/ili daju putem mleka (krave, ovce i koze), a zatim i količinu koja je potrebna da se proizvod preradi, upakuje (mleko, kiselo mleko, sir, ...), kao i da se uzme u obzir deo prinosa koji nije koristan (na primer saturacioni mulj kao otpad od šećerne repe, iznutrice iz životinja itd.). Detaljan algoritam izračunavanja virtuelne vode su opisali Mekonnen i Hoekstra (2010, 2012). Za potrebe ovog rada, iz pomenute publikacije su preuzete vrednosti SZV nekih proizvoda (tabela 2), koji su kasnije korišćeni u proračunu virtuelne trgovine vodom najznačajnijih proizvoda.

Tabela 2. Specifični zahtevi za vodom (SZV) proizvoda animalnog porekla.
Table 2. Specific water demand (SWD) of animal products.

Proizvod/*Product*	SZV/*SWD* (m^3/t)
Mleko/*Milk*	1020
Pileće meso/*Chicken meat*	4324
Puter/*Butter*	5553
Ulje i masti/*Oils and fat*	2364
Svinjsko meso/*Pork meat*	5988
Šećer/*Sugar*	1239

Izvor/*Source*: (Mekonnen i Hoekstra, 2010, 2012).

Rezultati i diskusija

Upoređivanjem SZV dobijenih našim proračunima i onima koje su prikazali gore pomenuti istraživači, jasno se vidi da nema velikih i značajnih odstupanja. Razlike se javljaju uglavnom kao posledica promene prosečnih prinosa, a u znatno manjoj meri potreba useva za vodom, što se naročito vidi na primeru graška ili luka, ili na osnovu prinosa dobijenih eksperimentalnim putem.

Izračunate vrednosti SZV dobijene na osnovu prinosa dobijenih eksperimentalnim putem i potrebe useva za vodom su značajno manje nego dobijene na osnovu prosečnih prinosa preuzetih iz statističkih godišnjaka. To ukazuje da se dobrim upravljanjem vodnim resursima može povećati efikasnost

korišćenja vode, da se značajna količina vode može zadržati u domicilnoj zemlji, te da to sve doprinosi održivosti vodnih resursa na nekom području, u državi, regionu ili na kontinentu.

Specifični zahev za vodom pšenice gajene u Srbiji u odnosu na Austriju, Nemačku, Mađarsku, Rumuniju i Sjedinjene Američke Države (SAD) je veći, što ukazuje da postoji prostor da se unapredi korišćenje vode podešavanjem sortimenta ili promenama u tehnologiji gajenja. Treba napomenuti da je situacija u vezi sa SZV pšenice u Srbiji povoljnija u odnosu na Rusiju, Italiju ili Grčku (tabela 3). Slični rezultati su dobijeni i za suncokret, s tim što su vrednosti SZV za Srbiju bolji nego vrednosti zabeležene u Rusiji, SAD i Rumuniji. Kod kukuruza postoji prostor za unapređenje proizvodnje, jer su u svim navedenim zemljama osim Rusije vrednosti SZV niže nego u Srbiji.

Tabela 3. Specifični zahtevi za vodom najvažnijih kultura koje učestvuju u spoljnotrgovinskoj razmeni nekoliko izabranih zemalja.
Table 3. Specific water demand of the staple crops, most frequently traded internationally.

Proizvod/*Product* Zemlja/*Country*	Pšenica/ *Wheat*	Kukuruz/ *Maize*	Suncokret/ *Sunflower*	Šećernarepa /*Sugar beet*	Soja/ *Soya bean* 120100	Krompir/ *Potato*	Jabuke/ *Apple*
Austrija/*Austria*	688	330	1133	58	1261	879	141
Nemačka/*Germany*	498	476	1173	71	1303	84	184
Grčka/*Greece*	3440	232	-	144	3435	364	440
Italija/*Italy*	2016	252	1843	145	1586	227	218
Mađarska/*Hungary*	1040	494	1966	89	1395	155	-
Rumunija/*Romania*	1338	874	2405	184	1848	228	993
Rusija/*Russia*	2384	1608	3586	215	4112	339	1133
SAD/*USA*	1302	377	2115	81	1380	778	252

Izvor/*Source*: (Chapagin i Hoekstra, 2004).

Specifični zahtevi šećerne repe i soje takođe mogu da se smanje, jer u zemljama sličnih zemljišnih i klimatskih karakteristika poput Austrije i Mađarske zahtevi su niži, što ukazuje da su naši prosečni prinosi niži. Na primer, kod krompira u našoj zemlji se SZV mogu umanjiti i do tri puta, ako se povećaju prinosi do nivoa koji se postiže na ogledima, dok se kod drugih kultura mogu umanjiti od 33% sve do 95% i izuzetno u nekim područjima do 300%.

Upoređivanje SZV kultura u odnosu na druge zemlje mora biti sveobuhvatno, jer nije uvek slučaj da visoki zahtevi za vodom potiču usled ekstenzivne poljoprivrede, već razlozi mogu biti i druge vrste na koje čovek ne može da utiče (kišniji region, moćnost i kapacitet zemljišta da zadrži vodu, toplotni režim). To se može manifestovati i na SZV životinjskog porekla (Ridoutt et al., 2012).

Uzimajući u obzir 100 najvažnijih proizvoda koji su bili u međunarodnom prometu, izračunate su virtuelne količine vode koje su izvezene iz Srbije i koje su uvezene za period 1995–1999. godine, odnosno 2010–2013. godine (tabela 4). Količina izvezene virtuelne vode u periodu 2010–2013. godine je povećana prosečno za 32%, dok se uvoz iste smanjio za tri puta. Ovakav trend je povoljan sve dok nije narušen vodni bilans jedne zemlje. U Srbiji, čak i sa dodatnim porastom izvoza poljoprivrednih proizvoda, neće doći do narušavanja vodne održivosti, jer je procenjeno da je za poljoprivredu raspoloživo $265.000 \cdot 10^6$ m^3vode.

Tabela 4. Upotrebljena količina vode za proizvodnju poljoprivrednih proizvoda koja je bila u spoljno-trgovinskoj razmeni (u 10^6 m^3vode).
Table 4. Water quantity used in agricultural production of internationally traded products (10^6 m^3).

Godina/ *Year*	Količina izvezene vode/ *Export quantity*	Količina uvezene vode/ *Import quantity*	Vodni bilans/ *Water balance*
2010	3068,9	342,7	2726,2
2011	2913,5	277,7	2635,8
2012	3386,8	348,9	3037,9
2013	3489,4	295,3	3194,1
1995–1999	2441,5	1763,6	677,9

Izvoz virtuelne vode izražene kroz monetarnu vrednost je takođe pozitivan, ali ne u odnosu kakav je kod zapremine vode. Naime, vrednost koja se ostvaruje izvozom vode varira od 404 10^6\$ do skoro 700 10^6\$ (tabela 5).

Tabela 5. Vrednost poljoprivrednih proizvoda u spoljnoj trgovini.
Table 5. The value of agricultural products in international trade.

Godina/ *Year*	Izvoz/ *Export*	Uvoz/ *Import*	Bilans/ *Balance*	10^6\$
2006				404
2007				658
2008				564
2010	696243	184029	512214	512,2
2011	899083	244382	654701	654,7
2012	978093	281590	696503	696,5
2013	834987	285865	549122	549,1

Trenutno stanje nije zabrinjavajuće, ali se već sada u mnogim zemljama rade analize radi podsticanja efikasnije potrošnje vode i uzgoja kultura koje troše manje vode, a mogu se plasirati na strana tržišta. Takva razmatranja su već rađena na primeru dve različite tehnologije gajenja u voćarstvu u Srbiji (Stričević et al., 2016;

Stričević et al., 2017), mlekarstva na Novom Zelandu (Zonderland-Thomassen i Ledgard, 2012), ili na primeru proizvodnje bioenergije, gde je ukazano koje je kulture povoljnije gajiti za proizvodnju biodizela, a koje zaproizvodnju bioetanola (Gerbens-Leenes et al., 2009).

Zaključak

Klimatske promene će uticati na potrebu efikasnije upotrebe vode u poljoprivredi, posebno zato što je poljoprivreda najveći korisnik slatke vode. Srbija trenutno više izvozi nego što uvozi virtuelne vode i to je nesumnjivo povoljno u ekonomskom smislu. Takođe, nema ugroženosti sa stanovišta samoodrživosti vodnih resursa. Međutim, dugororočna strategija treba da ide u pravcu mogućnosti povećanja izvoza poljoprivrednih proizvoda, a da se pri tome ne poveća izvoz virtuelne vode. To se može obezbediti unapređenjem poljoprivredne proizvodnje putem izbora sortimenta, tehnologije gajenja i načina korišćenja vode.

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THE AGRICULTURAL WATER FOOTPRINT AND ASSESSMENT OF VIRTUAL WATER TRADE. DOES SERBIA IMPORT OR EXPORT WATER?

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A b s t r a c t

Limited water resources, an increase in water demand and a changing climate triggered the development of new concepts for assessment of water demand and water consumption locally and globally. The newest concepts that successfully tackle this issue are water footprint and virtual water trade. Aims of this study are: (1) to define specific water demand for the most important agricultural products in the international trade of the Republic of Serbia, (2) to assess possibilities of an increase in water productivity for those products, and (3) to assess sustainability of water resources in Serbia, based on the ratio of import/export during virtual water trade. Specific water demand for wheat, maize, sunflower, sugar beet and soya bean has been calculated and compared with specific water demand in other countries. Results prove that water productivity can be improved by, for example, using other varieties of crops or modifying cultivation technology. The ratio of imported/exported virtual water quantities for the periods 1995–1999 and 2010–2013 in Serbia shows that more water was exported than imported. Sustainability of water resources in Serbia will not be endangered even if the export of agricultural products is increased.

Key words: specific water demand, virtual water trade, agricultural products, water productivity.

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ULTRASOUND-ASSISTED EXTRACTION OF SUNFLOWER OIL FROM THE CAKE AFTER SUNFLOWER SEED PRESSING

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Abstract: The influence of ultrasound application on the extraction (UE) of the cake after sunflower seed pressing was studied. Three different solvents were used for extraction in the Soxhlet apparatus: n-hexane, petroleum ether and extraction petrol. Petrol for extraction showed the highest yield. It has been shown that the using of ultrasound improved the extraction kinetics in the initial period – washing, and in the second period – the diffusion of oil from the mass of the cake. The parameters of the non-stationary diffusion model with and without the use of ultrasound were k' = 0.039 min^{-1}, k' = 0.026 min^{-1} and b' = 0.713, i.e. b' = 0.589, respectively. Oil yield was also slightly higher in the ultrasonically supported extraction.

Key words: ultrasound, sunflower, extraction.

Introduction

The production of sunflower oil is one of the most characteristic examples of the application of extraction on an industrial level. Therefore, this process is well developed and is the subject of research in many studies (Baümler et al., 2016; Rai et al., 2016). The production of sunflower oil involves several stages: separating the grain from impurities, moistening the grain in the water vapor for easier separation of the shell, pressing, extracting the residual oil from the cake, and final treatment of the oil (dearomatization and decolorization). The cake, after extraction, contains a certain amount of residual oil and as such is used for the animal food production. Preferably, the oil content of the cake after extraction at an acceptable time interval is as low as possible.

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The using of ultrasound, with known effects of heterogeneous systems (cavitation), can have a positive effect on the extraction kinetics and yield (Amirante et al., 2017; Zhang et al., 2017). Many results of the studies in the field of extraction confirm the following: extraction of oil from the fennel seeds *(Foeniculum vulgare L.)* (Moubarik et al., 2011), mandarin orange *(Citrus reticulata)* (Ma et al., 2008), rose hip (*Rosa canina L.*) (Szentmihaályi et al., 2002) as well as sunflower seeds (*Helianthus annuus L.*), (Zardo et al., 2017), tobacco *(Nicotiana tabacum L.*) (Stanisavljević et al., 2009) and sage (*Salvia officinalis L.*) (Veličković et al., 2006; Milenović et al., 2002).

The results presented in this study are preliminary research results obtained within the framework of the project for improving the efficiency of the technological process of extracting sunflower oil in the oil company "Plima" in Kruševac. The goal of the project was to find out the rationality of the adaptation of the existing equipment.

Materials and Methods

Materials

As an extraction material in this study, a cakeof the mechanically pressed sunflower seeds was used. The crushing of the cake was done in an electric grinder with rotary blades (15 000 rpm.). The resulting particles were then separated through standard sieves. For the extraction, a fraction from 0.4 mm to 0.5 mm was used. The content of moisture in the samples is given in Table 1. The n-hexane, petrol ether and high-purity petrol were used for the extraction.

Table 1. The moisture content in the samples, % m/m.

Sunflower seed	Cake before extraction	Cake after extraction
7.31	4.82	13.30

Extraction in a Soxhlet apparatus (SA)

In this study, two extraction techniques were applied. The ultrasound-assisted extraction in a modified Soxhlet apparatus (SA) (Luque-Garcıa and Luque de Castro, 2004), as shown in Figure 1, then a classical batch reactor for extraction with stirring, and an ultrasonic generator (BE) were used in this research.

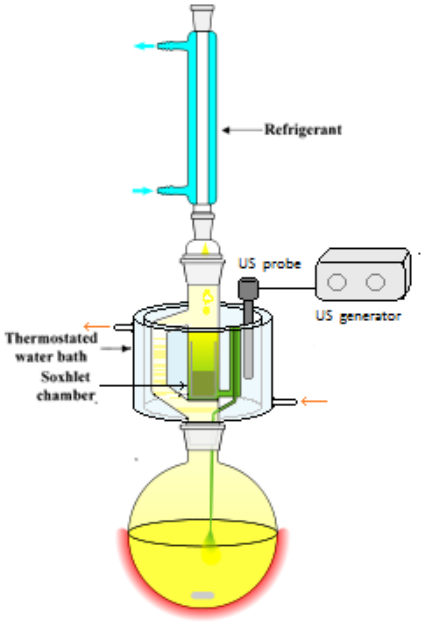

Figure 1. A modified Soxhlet apparatus for ultrasound-assisted extraction (Luque-Garcıa and Luque de Castro, 2004).

The (SA) technique with and without the use of ultrasound was used to determine the maximum yield. The technique (BE) with and without ultrasound was used to monitor the extraction kinetics. For the (SA) technique, a sample of 10 g and 100 mL of the appropriate solvent was used. The extraction time was 6 hours (24 cycles). After the final extraction, the solvent was eliminated in a vacuum evaporator at 50°C.

Extraction in the batch reactor

The extraction kinetics in this study was investigated in a classical batch reactor with stirring and the ultrasonically assisted extraction in the batch reactor. In both cases, high-purity petrol was used as a solvent. A 1:10 hydromodule was applied, and a sample of 5 g of the sunflower cake was placed in an Erlenmeyer of 100 mL with 50 mL of the solvent. The set-up consisted of an ultrasonic cleaner (EI, Niš, Serbia; total nominal power: 2 x 50 W; and internal dimensions: 300 x 220 x 155 mm), operating at 40 kHz frequency. The Erlenmeyer was then immersed in an ultrasonic bath filled with water up to 1/3 height. The operating temperature was constant at 25^{o}C (±0.2^{o}C). The extraction times were 2.5 min, 5 min, 10 min, 20 min, 40 min and 60 min. After completion of the extraction, the solvent was removed in a vacuum evaporator at 50°C.

Results and Discussion

Extraction in the Soxhlet apparatus (SA)

The results of the sunflower oil extraction from the pressed sunflower seeds are shown in Table 2 and Figure 2. There was a positive contribution to the use of ultrasound. Compared to n-hexane and petrol ether, petrol showed the best yield, both without and with ultrasound. The contribution of ultrasound to all samples ranged from 1% to 1.5%.

Table 2.The sunflower oil residue in samples after pressing in operating conditions, determined without and with ultrasonically assisted extraction, % m/m.

	n-hexane		Petrol ether		Petrol for extraction	
	Without ultrasound	With ultrasound	Without ultrasound	With ultrasound	Without ultrasound	With ultrasound
1	19.02	19.97	19.50	20.67	19.73	21.11
2	21.95	23.05	22.31	23.65	22.44	24.01
3	20.01	21.01	20.08	21.29	20.19	21.60

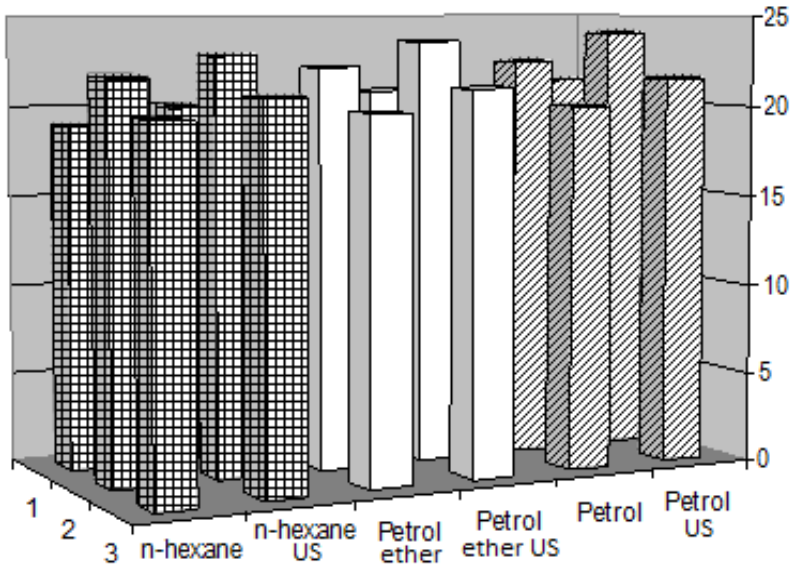

Figure 2. A graphic presentation of ultrasound application in sunflower oil extraction of the mechanically pressed sunflower seeds in various solvents.

Extraction in the batch reactor (BE)

The results of the sunflower oil extraction kinetics, with and without the use of ultrasound, are shown graphically in Figure 3. In both cases, the shape of the curve is as in the extraction of tobacco oil (Stanisavljević et al., 2009), the extraction of sage (Veličković et al., 2006) and St. John's wort *(Hypericum perforatum)*(Milenović et al., 2002). The kinetic curve had two distinctly separate periods. The first part (<5 min), flushing, was characterized by a rapid change in the concentration of oil in the solvent, and the second part, the period of slow extraction (difussion phase), in which the slow progress in the yield was observed. The largest part of the oil (> 95%) was extracted for up to 20 minutes and complete extraction was achieved in 60 minutes.

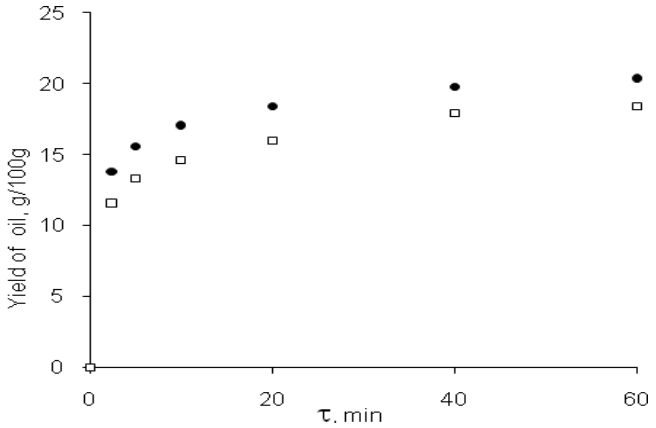

Figure 3. Kinetics of sunflower oil extraction from the cake aftersunflower seed pressing with (∗) and without (□) the use of ultrasound.

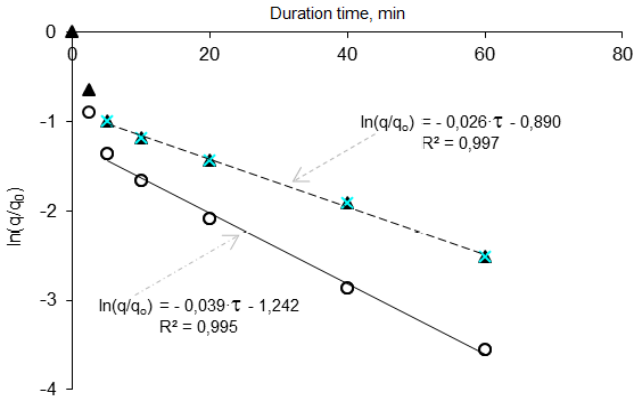

Figure 4. The linear shape of the kinetic curve of the extraction of sunflower oilwith (o) and without (Δ) ultrasound.

Table 3. Parameters of the unsteady diffusion model without and with ultrasuond.

Without ultrasound			With ultrasound		
k', min^{-1}	b'	R	k', min^{-1}	b'	R
0.026	0.589	0.997	0.039	0.713	0.995

A large number of models can be found in the literature describing solid-liquid extraction. However, the kinetics of the extraction process of substances from raw materials has most often been modeled using the unsteady diffusion through plant material (Stanković et al., 1994), the film theory (Pekić et al., 1988) and the empirical equation of Ponomaryov (1976). Some of them are shown in Table 4.

Table 4. Some mathematical models describing solid-liquid extraction.

Model	Equation	Linearized form	References
Ponomaryov	$(q_o-q)/q_o= b+kt$		Ponomaryov (1976)
Film theory		$\ln(1 - c / c_o) = \ln(1 - b') - k'\tau$	Pekić et al. (1988)
Non-stationary diffusion model	$q / q_o = (1 - b') \cdot e^{-k'\tau}$	$\ln(q / q_o) = \ln(1 - b') - k'\tau$	Stanković et al. (1994)
Parabolic diffusion model	$\bar{q} = A_o + A_1\tau^{1/2} + A_2\tau$		Kitanović et al. (2008)
Power law model	$\bar{q} = B\tau^n$	$\ln \bar{q} = \ln B + n \ln \tau$	Kitanović et al. (2008)
Hyperbolic model	$\bar{q} = \frac{C_1\tau}{1 + C_2\tau}$	$\frac{1}{\bar{q}} = \frac{1}{C_1} \cdot \frac{1}{\tau} + \frac{C_2}{C_1}$	Kitanović et al. (2008)
Weibull's equation	$\bar{q} = 1 - \exp\left[-\left(\frac{\tau}{\delta}\right)^m\right]$	$\ln[-\ln(1 - \bar{q})] = -\ln D + m \ln \tau$	Kitanović et al. (2008)
Elovich's equation	$\bar{q} = E_o + E_1 \ln \tau$		Kitanović et al. (2008)

In this paper, a model based on the unsteady diffusion (non-stationary diffusion model) is used, which has the following form:

$$q/q_o=(1- b')\cdot\exp(-k'\tau) \quad (1)$$

or a linearized form:

$$\ln(q/q_o)=\ln(1- b') - k'\tau \quad (2)$$

where: b' is the washing coefficient, 1; k' is the slow diffusion coefficient, min^{-1}, q is the oil content in the seeds during the extraction, g/100 g; q_o is the oil

content initially present in the seeds, g/100 g, and τ is time in minutes. The b'and k' were determined experimentally from the slope and segment by plotting $\ln(q/q_o)$ against τ. The linear regression analysis, in this work, was done using Microsoft Office Excel 2007.

The model is based on the two-stage extraction mechanism and two parameters included. The first parameter (b') characterizes the washing stage (the so-called washing coefficient) and the second parameter (k') characterizes the slow extraction (the so-called slow extraction coefficient). A graphic presentation of a change of the $\ln(1-q/q_o)$ during the time is shown in Figure 4. Significant deviations from the model equation could only be observed in the initial period. The values of both kinetic parameters are presented in Table 3.

On the basis of linear forms of the kinetic curves (shown in Figure 4) and the linear correlation coefficient, it can be concluded that the kinetic models applied fitted well the experimental data. The coefficients of linear correlation were higher than 0.995 based on values in Table 3. In the second stage of extraction, a high level of coefficients of linear correlation (R) illustrates a very good agreement between the theory model and the experiment.

Based on the values of the rapid (b') and slow (k') parameters of the extractions shown in Table 3, the positive effect of ultrasound is seen. The coefficient of slow extraction without ultrasonic application was $k' = 0.026\ min^{-1}$, whereas with the use of ultrasound $k' = 0.039\ min^{-1}$. The coefficient of slow extraction was found to be 50% higher with the use of ultrasound. The effect of ultrasound is probably due to cavitation. The cavitation phenomenon is likely to partially destroy the structure of the solid phase (Toma et al., 2001). In addition, the cavitation phenomenon is likely to partially increase the temperature of oil in the solid phase. This affects the viscosity of the oil as well. In this way, the diffusion of the oil through the solid phase is accelerated.

The coefficient of washing without the application of ultrasound was b' = 0.589, whereas with the use of ultrasound b' = 0.713. The use of ultrasound increased the rate of extraction in the initial period (washing) by 21%. Obviously, a greater contribution of the ultrasound to the extraction of the oil was recorded in a slower phase, in the diffusion, through the solid material.

Conclusion

The obtained results show that the ultrasound had a positive effect on extraction in the Soxlet apparatus. For the same number of cycles in the Soxlet apparatus, the yield with ultrasound was about 1.5% higher. In relation to n-hexane and petroleum, the extraction with petrol showed the best results (yield) in extraction, both without and with ultrasound.

A two-parameter mathematic model was used for the extraction kinetics modeling; a model based on unsteady diffusion through solid material. In the second stage, the applied mathematical model best followed experimental results. The linear correlation coefficient was R = 0.995 without ultrasound and R = 0.997 by using of ultrasound. A positive effect of ultrasound, both on the extraction rate and on the oil yield, was confirmed by the applied model (b', k'). As is known, in addition to ultrasound, many other parameters (grain size, temperature, hydrodynamic conditions, etc.) also affect the extraction efficiency. Therefore, the obtained results provide a good base for further research.

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ULTRAZVUČNO POTPOMOGNUTA EKSTRAKCIJA POGAČE NAKON PRESOVANJA LJUŠTENIH ZRNA SUNCOKRETA

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R e z i m e

U radu je istražen uticaj primene ultrazvuka na ekstrakciju pogače nakon presovanja oljuštenih zrna suncokreta. Za ekstrakciju u Soxlet aparatu su primenjena tri rastvarača: n-heksan, petrol etar i ekstrakcioni benzin. Kao najefikasniji rastvarač pokazao se ekstrakcioni benzin. Doprinos primene ultrazvuka na ekstrakciju u Soxlet aparatu, kod sva tri rastvarača je 1% do 1,5% pri istom broju ciklusa, 24.

Kinetika procesa, bez i sa primenom ultrazvuka, praćena je u šaržnom reaktoru uz primenu ekstrakcionog benzina. Koeficijent linearne korelacije (R=0,995) potvrđuje dobro slaganje eksperimentalnih rezultata i primenjenog modela nestacionarne difuzije kroz čvrst materijal. Primena ultrazvuka pri ekstrakciji u šaržnom reaktoru pokazala je pozitivan efekat celim tokom procesa, kako u početnom periodu (ispiranje) tako i u drugom delu – spora ekstrakcija. Pozitivan efekat primene ultrazvuka, kako na brzinu tako i na prinos, potvrđuje se vrednostima parametara primenjenog modela nestacionarne difuzije (b', k'). Parametri primenjenog modela sa i bez ultrazvuka su k' = 0,039 min^{-1} i k' = 0,026 min^{-1} odnosno b' = 0,713 i b' = 0,589. Na osnovu ovih parametara, doprinos primene ultrazvuka u istraživanom slučaju veći je u fazi spore ekstrakcije – difuzije za 50% u odnosu na fazu ispiranja, gde je doprinos 21%.

Dobijeni rezultati ukazuju na opravdanost daljih istraživanja uticaja i drugih relevantnih parametara (temperatura, hidromodul, veličina čestica itd.) na efikasnost primene ultrazvuka pri ekstrakciji ulja iz pogače nakon mehaničkog presovanja zrna suncokreta.

Ključne reči: ultrazvuk, suncokret, ekstrakcija.

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THE EXTRACT OF FENNEL FRUIT AS A POTENTIAL NATURAL ADDITIVE IN FOOD INDUSTRY

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Abstract: In this study, the polyphenol profile and antioxidant activity of the hydro-ethanolic extract of the fennel fruit were examined in order to investigate the possibility of its application as a potential functional food additive. Total phenols were analyzed by the method of Folin-Ciocalteu, while total flavonoids were determined by the aluminum chloride colorimetric method. The separation and quantification of phenolic compounds were performed by LC-MS/MS analysis, using a multiple reaction monitoring (MRM) mode. The antioxidant capacity was determined by FRAP and DPPH assays.

The high values of total phenolics and flavonoids were found, as well as high antioxidant activity which amounted to 9023.33 ± 38.19 μmol Fe(II)/l and 3.73 ± 0.04 mmol TE/l, tested by FRAP and DPPH assays, respectively. Among the identified phenolic compounds, *p*-hydroxybenzoic and chlorogenic acids were detected as predominant. The obtained results indicated that the hydro-ethanolic extract of the fennel fruit can be used in food industry as a potential natural antioxidant.

Key words: fennel fruit, hydro-ethanolic extract, antioxidant activity, total phenols, LC/MS.

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Introduction

The application of herbs in the prevention and treatment of various diseases is as old as mankind itself. Over time, herbal compositions in the widespread traditional use have become an integral part of modern pharmacotherapy and conventional modes of treatment. Similarly, the connection between diet and health has been known since ancient times. Medicinal plants have found their place not just in the field of health preservation, but also in the food and beverage industry as their functional addition. Recent findings in the fields of biology and medicine have confirmed the hypothesis that diet plays a decisive role in the modulation and control of various body functions and in achieving and maintaining good health.

On the basis of this, the concept of functional food was developed and also a new scientific discipline, known as functional food science. Functional food means any food that, in addition to its nutritional value, contains ingredients which have positive effects on human health and reduces the risk of the disease (Kim et al., 2006). Medicinal plants, as a rich source of various bioactive compounds, exhibiting beneficial effects on human health have largely found their place in the development of functional food.

Fennel (*Foeniculum vulgare* Mill.) is a highly regarded medicinal and aromatic plant from the *Apiaceae* family. It is widespread in the Mediterranean, but it is grown in many countries of the world as well. Recent research has shown that *F. vulgare* has different pharmacological properties such as anti-allergic, analgesic, anti-inflammatory, antioxidant, antibacterial, anti-cancer, anti-stress, cytotoxicity, etc. (Kooti et al., 2015). Fennel is a highly valued spice plant. The whole plant has a very intense specific odor, and in many countries, it is cultivated and consumed as a vegetable, especially its succulent young sprouts. The fruit of fennel is used in cooking as a flavoring and odorant agent and in food industry and confectionery as well as for the production of herbal liqueurs and spirits (Timasheva and Gorbunova, 2014).

The essential oil of fennel was largely examined, both from the aspects of its chemical composition and its pharmacological activities. Various growing localities have an impact on the qualitative and quantitative composition of the fennel essential oil (Piccaglia and Marotti, 2001; Aćimović et al., 2015). Likewise, different stages of fruit maturation have a significant influence on the yield and chemical composition of the sweet fennel essential oils (Telci et al., 2009). Apart from analyzing the chemical profile of essential oils, a very strong antibacterial effect on the common foodborne pathogens was established (Dadalioğlu and Evrendilek, 2004). The high antifungal and antioxidant potential of the fennel essential oil was also proven (Singh et al., 2006).

Furthermore, extracts of the fruit of fennel, prepared with different methods and different extraction agents, were examined from the aspects of their chemical composition and pharmacological activity (Kooti et al., 2015). Phenolic compounds and antioxidant activity of water and methanolic extracts (Cai et al., 2004), and extracts prepared with 80% methanol (Surveswaran et al., 2007) were examined. In the study of De Marino et al. (2007), phenolic glycosides and antioxidant activity were analyzed from the methanolic extracts of the fennel fruit. The methanolic extracts of fennel seeds were also evaluated from the points of view of their antioxidant and anti-carcinogenic effects (Mohamad et al., 2011). The antioxidant potential of methanolic extracts of different parts of the fennel plant was also tested (Barros et al., 2009) as well as total phenols and antioxidant capacity of water infusion made of numerous medicinal plants including the fennel fruit (Katalinić et al., 2006).

From a toxicological point of view, acetone, methanol and other organic solvents are not suitable as solvents for the preparation of extracts used orally. In contrast to them, hydro-ethanolic extracts may be used in the production of pharmaceutical compositions in the form of plant drops or solutions, and also in the food industry. As there is no information on *in vitro* studies of the polyphenol content and the antioxidant activity of hydro-ethanolic extracts of fennel fruits, the goal of this study was to investigate the antioxidant activity of the extract obtained by extraction with a mixture of water and ethanol (50:50), and assess the possibility of using this extract as a potential source of natural antioxidants and functional food additives.

Materials and Methods

Preparation of extract

The fennel fruit was purchased in dried form in the pharmacy of the Institute of Medicinal Plants Research "Dr JosifPančić", Belgrade (serial number: 3580611). The extract of the fennel fruit was prepared by double percolation using a modified pharmacopoeia method, in a glass percolator at room temperature, using a 50% ethanol-water solvent, where the ratio of plant material to the resulting extract was 1:1 (Pharmacopoea Jugoslavica, 1984).

Dry matter and pH

The pH value of the tested extract was determined by a pH meter with the glass electrode (WTW inoLab), whereas the soluble dry matter (DM%) was determined using a refractometer (Gramma Libero).

Determination of total phenol content

The total phenol content (TPC) was determined using Folin-Ciocalteu reagent and expressed as a gallic acid (GA) equivalent (mg GAE)/l of the extract (Fu et al., 2011).

Determination of total flavonoid content

The content of the total flavonoids in the extract was determined by the spectrophotometric method, based on the production of complex compounds of flavonoids with aluminium chloride. The standard solution of quercetin in ethanol was used as a referent sample, and the results were expressed as mg of equivalent per liter of the extract of quercetin (Verzelloni et al., 2007).

Antioxidant activity

FRAP assay: Total antioxidant activity was investigated using the ferric reducing antioxidant power (FRAP) assay, which is based upon reduction of Fe(III) - TPTZ in acidic conditions (Fu et al., 2011). The standard curve was constructed using the $FeSO_4$ solution, and the results were expressed as μmol Fe(II)/l of the extract.

DPPH radical assay: Radical scavenging activity of the tested extract was determined by the DPPH method (Jakobek et al., 2007). The trolox solution was prepared as a reference standard and the results were presented as the TE mmol/l of extract.

LC analysis

The separation of phenolic compounds in the extract was carried out using a Waters Acquity UPLC system equipped with a photodiode array (PDA) detector, and interfaced to a mass spectrometer with the triple quadrupole analyser (QqQ). The separation column was a ZORBAX Eclipse XDB C18 column (150 × 4.6 mm; 5 μm). The LC conditions were as following: mobile phase flow rate of 0.7 ml/min, column temperature of 25°C, injection volume of 1 μl, and the solvent gradient (time [min]/ solvent B (%): 0/5, 20/16, 28/40, 32/70, 36/98, 45/98, 46/5, 55/5), where solvent A was the 0.2% solution of formic acid in deionized water (v/v) and solvent B was acetonitrile. The PDA detector range was 190–450 nm. The hydro-ethanolic extract of the fennel fruit was filtered through Premium Syringe Filters, Regenerated Cellulose 0.45 μm, 15-mm filters, before injection.

MS/MS-MRM analysis

The identification of phenolic compounds was performed by comparing their retention times (t_R), UV spectra and multiple reaction monitoring (MRM) transitions with those of reference standards (Table 1). The quantification of the identified compounds was performed using the external standard method. The conditions under which the electrospray ionization (ESI) source operated were: negative ionization mode, source temperature of 150°C, desolvation temperature of 350°C, desolvation gas flow rate of 800 l/h, capillary voltage of 3.5 kV, extractor voltage of 3 V, cone voltage of 30 V (60 V for rutin), and collision energy of 9 eV (20 eV for rutin) with argon used as a collision gas. Mass Lynx 4.1 software was used for data acquisition and peak integration.

Table 1.The parameters for the identification and quantification of phenolic compounds in the hydro-ethanolic extract of the fennel fruit.

Phenolic compound	Molecular formula	Molar mass	MRM transition	t_R (min)	UV_{max} (nm)
Protocatechuic acid	$C_7H_6O_4$	154	153→109	9.14	218, 260
p-hydroxybenzoic acid	$C_7H_6O_3$	138	137→93	13.81	255
Chlorogenic acid	$C_{16}H_{18}O_9$	354	355→163	14.33	246, 325
Caffeic acid	$C_9H_8O_4$	180	179→135	17.06	243, 323
p-coumaric acid	$C_9H_8O_3$	164	165→91	23.34	230, 309
Ferulic acid	$C_{27}H_{30}O_{16}$	610	193→134	25.10	278, 321
Rutin	$C_{27}H_{30}O_{16}$	610	609→301	24.64	253, 341

MRM − multiple reaction monitoring, t_R− retention time, UV_{max} − ultraviolet maximum.

Statistical analysis

All measurements were done in triplicate and results were expressed as mean ± standard deviation.

Results and Discussion

The hydro-ethanolic extract of the fennel fruit was obtained using the traditional percolation method. The soluble solids of this extract measured using a refractometer were 23%, whereas pH was 6.3. The amount of total flavonoids and total phenolic content of the tested extract was 1524.86 ± 4.81 mg (quercetin equivalents) QUE / l and 1534.97 ± 6.26 mg GAE / l of the extract, respectively (Table 2).

Oktay et al. (2003) obtained the higher value of total phenolic content in the ethanol extract of fennel seeds compared to the corresponding water extract (90.00 and 21.25 μg GAE / 1mg of the ethanol and water extracts of fennel seeds, respectively).

In the hydro-methanolic extract of fennel shoots, the significant amounts of flavonoids and high content of phenols were determined (De Marino et al., 2007).

Table 2. TPC, TFC and antioxidant activity of the extract of the fennel fruit.

Parameter analyzed	Value
TPC (mg GAE/l)	1534.97 ± 6.26
TFC (mg QUE/l)	1524.86 ± 4.81
FRAP (μmol Fe(II)/l)	9023.33 ± 38.19
DPPH (mmol TE/l)	3.73 ± 0.04

TPC − total phenolic content, TFC − total flavonoid content, FRAP − ferric reducing ability of the plasma method, DPPH − 2,2-diphenyl-1-picrylhydrazil.

The antioxidant capacity of our extract was 9023.33 ± 38.19 μmol Fe (II)/l and 3.73 ± 0.04 mmol TE/l, tested by FRAP and DPPH assays, respectively (Table 2). A high antioxidant capacity has also been reported in the work of Cai et al. (2004) in hydro and methanolic extracts of the fennel fruit (TEAC: 150.6 and 105.9 μmoltrolox/100g DW, respectively). In addition, the methanolic extract of fennel seeds investigated in the work of Surveswaran et al. (2007) exhibited even a stronger potential (DPPH: 2.63 mmol/100 g DW; FRAP: 1.79 μmol/g DW) compared with our results. The antioxidant activity of hydro and ethanolic extracts of fennel seeds was also evaluated by different antioxidant methods compared to standard antioxidants such as BHA, BHT, and α-tocopherol (Oktay et al., 2003). It has already been reported that the antioxidant capacities of different extracts have a strong relationship with the solvent combination used, mostly due to the different antioxidant capacities of compounds with different polarities (Boeing et al., 2014).

In the study of Cai et al. (2004), a very significant positive correlation between the content of polyphenolic compounds and antioxidant capacity was found (R^2 values – 0.950), indicating that polyphenolic compounds had the greatest contribution to the activities of the plant antioxidants. In addition, a similar correlation has been shown in the work of Surveswaran et al. (2007), with R values: TPC-ABTS 0.9690; TPC-DPPH 0.9378; TPC-FRAP 0.8941.

Infusions of the 70 different medicinal plants have been tested for antioxidant capacity and total phenols and it was found that these parameters were lower for the infusion made of the fennel fruit compared to other tested plants (*Foeniculi fructus*: total phenolics− 29 mg CE/l; FRAP 142 μmol/l) (Katalinić et al., 2006). However, significantly higher values were observed in our sample in relation to those values, which may be attributed to the higher solubility of the essential oil in ethanol than in water (infusion). It has already been verified that the essential oils distilled from the fennel fruit possess a high antifugal and high antioxidant capacity (Singh et al., 2006). The essential oil was

found 100% antifungal against *Aspergillus niger*, *A. flavus*, *F. graminearum* and *F. moniliforme*.

In order to confirm that the phenolic compounds were responsible for the antioxidant activity of the hydro-ethanolic fennel fruit extract, the identification and quantification of phenolic compounds by the LC-MS/MS method in a multiple reaction monitoring (MRM) mode were performed. Extracted MRM chromatograms of quantified phenolic compounds of the tested extract are shown in Figure 1.

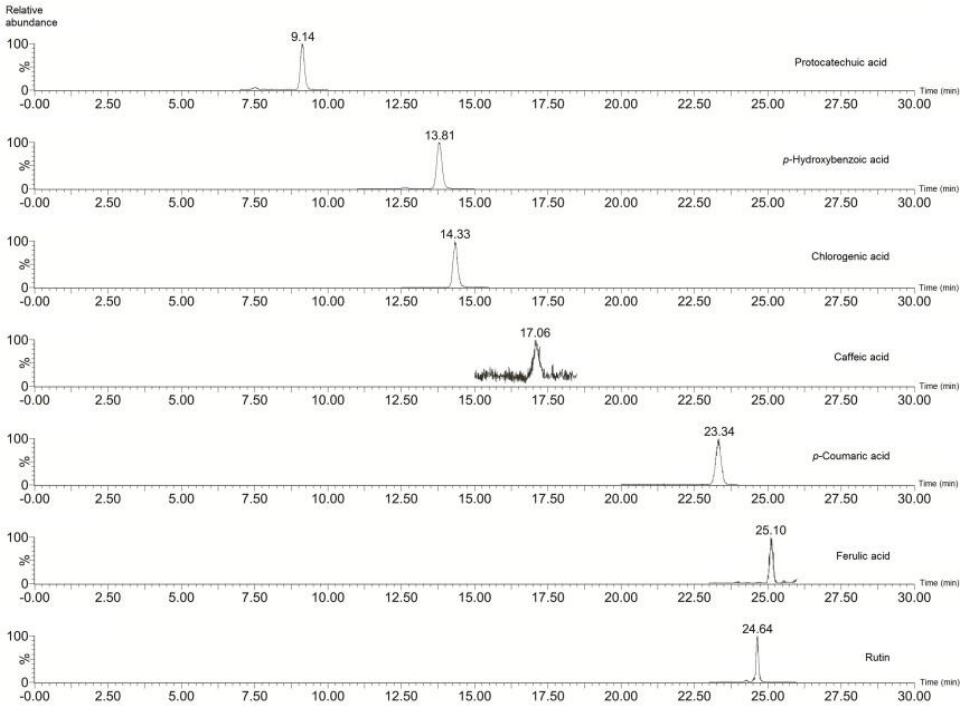

Figure 1. MRM chromatograms of protocatechuic, *p*-hydroxybenzoic, chlorogenic, caffeic, *p*-coumaric, and ferulic acids and rutin, respectively in the extract of the fennel fruit recorded in the (-) ESI mode.

As can be seen in Table 3, *p*-hydroxybenzoic, chlorogenic, *p*-coumaric, ferulic, caffeic acids, rutin and protocatechuic acid were identified, whereas gallic and ellagic acids, epicatechin, quercetin, myricetin and kaempferol as well as cinnamic and vanillic acids were not detected although these compounds were reported in the studies of other authors (Cai et al., 2004; Parejo et al., 2004).

On the other hand, a high amount (62.99 mg/l) of *p*-hydroxybenzoic acid was detected, as well as a significant amount of chlorogenic and p-coumaric acids. The esters of *p*-hydroxybenzoic acid are used as preservatives in pharmaceuticals, cosmetics and food industry (Aalto et al., 1953). The appreciable amounts of this acid detected in our sample indicated that this extract seemed to have a potential preservative effect. Different concentrations and isomeric mixtures of chlorogenic acid are present in coffee, wine, vegetable and fruit juices and in various herbal infusions, and it has been suggested that the consumption of these beverages can reduce the risks of different chronic diseases (Liang et al., 2016). Likewise, the antioxidant activity of ferulic, caffeic and chlorogenic acids has already been confirmed in an *in vitro* study (Milić et al., 2000).

Table 3. The content of phenolic compounds in the hydro-ethanolic extract of the fennel fruit (mg/l of extract).

No.	Compound	Content±SD (mg/l)
1.	Protocatechuic acid	4.04±0.18
2.	*p*-hydroxybenzoic acid	62.99±3.42
3.	Chlorogenic acid	30.82±1.55
4.	Caffeic acid	1.31±0.06
5.	*p*-coumaric acid	11.25±0.73
6.	Ferulic acid	6.12±0.82
7.	Rutin	6.81±0.34

The presence of chlorogenic, ferulic, caffeic acids as well as of rutin, quercetin and kaempferol was reported in the extracted residue (waste) after the production of the essential oil of the fennel from the period of flowering (Boeing et al., 2014), whereas the latter two compounds were not recorded in our sample. Differences among the detected compounds of various fennel extracts can occur due to the fact that the different fennel plant genotypes, grown in different climate conditions, were used for the extractions. Similarly, extractions were performed using different parts of the fennel plant and using different methods and solvents.

The obtained results indicate that the investigated extract represents a good source of polyphenolic compounds and can be used in food industry as a potential source of natural antioxidants.

Conclusion

The main advantage of the application of the hydro-ethanolic extract of the fennel fruit compared to the methanolic or acetone extract is the fact that it can be used in food industry without the removal of the solvent. In this work, high values of phenols and flavonoid compounds as well as an effective degree of the

antioxidant capacity of the hydro-ethanolic extract of the fennel fruit have been obtained. The various phenolic acids were recorded, with *p*-hydroxybenzoic and chlorogenic acids as the most dominant. This extract can be used in the food industry (production of alcohol and non-alcohol beverages or confectionery), not only as a specific flavoring, but also as an effective and easily accessible source of natural antioxidants with a potential preservative effect.

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EKSTRAKT PLODA MORAČA KAO POTENCIJALNI PRIRODNI ADITIV U PREHRAMBENOJ INDUSTRIJI

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R e z i m e

U ovom radu određivan je sadržaj polifenola i antioksidativna aktivnost vodeno-etanolnog ekstrakta morača, sa ciljem ispitivanja mogućnosti njegove primene kao potencijalnog funkcionalnog aditiva. Ukupni fenoli su analizirani metodom po Folin-Ciocalteu, dok je ukupan sadržaj flavonoida određen kolorimetrijskom metodom primenom aluminijum hlorida. Razdvajanje i kvantifikacija fenolnih jedinjenja postignuti su upotrebom LC-MS/MS metode u režimu koji omogućava istovremeno praćenje više jonskih prelaza. Antioksidativni kapacitet je određivan primenom testova FRAP i DPPH.

U testiranom ekstraktu dobijene su visoke vrednosti za ukupne fenole i flavonoide, a dobijena je i visoka vrednost antioksidativne aktivnosti, koja je iznosila 9023.33 ± 38,19 mmol Fe(II)/l i 3,73 ± 0,04 mmol TE/l, računato primenom testa FRAP odnosno testa DPPH. Među fenolnim jedinjenjima, *p*-hidroksibenzoeva i hlorogena kiselina su pronađene kao dominantne. Dobijeni rezultati ukazuju na to da se ekstrakt morača može primenjivati u prehrambenoj industriji kao potencijalni prirodni antioksidans.

Ključne reči: morač, vodeno-etanolni ekstrakt, ukupni fenoli, antioksidativna aktivnost, LC/MS.

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ECONOMIC IMPACT AND DETERMINANTS OF ADOPTION OF IMPROVED MAIZE PRODUCTION TECHNOLOGIES

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Abstract: The problem of what production technologies to adopt, and the degree to which farm operations should be improved for attainment of optimum economic benefit have remained undetermined. This study analysed the economics and determinants of adoption of the improved maize (*Zea mays*) production technology package in Oyo State of Nigeria. A multistage sampling procedure was employed to select one hundred and twenty maize producing farmers for the study in 2016. Data for the study were collected using a structured questionnaire and analysed with descriptive statistics and adoption index, regression analysis and the standard enterprise budgetary analysis. Results from the regression analysis showed that variables such as sex, farming experience, years of education, extension visits, and level of awareness of the technologies had a significant and positive influence on the adoption of improved maize technologies in the study area. Findings from the budgetary analysis revealed that improved maize production technology adopters made ₦438,367.23 compared to ₦374,426.44 profits per hectare of maize produced by the non-adopters during the year of survey. The results further revealed that on every naira invested in maize production, the adopters were able to make ₦7.64 in return compared to ₦6.00 returns by the non-adopters. There is the need for an increase in awareness of maize production technologies among the farmers, through the extension agents and social networks in order to increase the level of adoption of maize technologies.

Key words: adoption index, economic analysis, maize production, maize farmers.

Introduction

A fundamental source of agricultural transformation is technological change, which accompanies the introduction of modern agricultural technology and improved cultivation practices in the context of developing countries, such as sub-

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Saharan Africa (Otsuka, 2016). Increasing agricultural productivity using improved agricultural technologies that enhance sustainable food and fibre production is critical for sustainable food security and economic development (Mwangi and Kariuki, 2015). This study considered maize production technologies because maize is the most widely-grown staple food crop in sub-Saharan Africa (SSA) occupying more than 33 million hectares each year (FAOSTAT, 2015). The crop covers nearly 17% of the estimated 200 million hectares of cultivated land in sub-Sahara Africa, and is produced in diverse production environments and consumed by people with varying food preferences and socioeconomic backgrounds. Nigeria has been reported as the tenth largest producer of maize in the world, and the largest maize producer in Africa (IITA, 2012). Maize has grown from what used to be a back yard crop in the forest zone to a largely commercial crop grown now mostly in the savannahs of Nigeria. It is grown all over the country but concentrated in Oyo, Kwara, Niger, Kaduna, Nasarawa, Bauchi, Plateau, Taraba, Gombe and Adamawa (FMARD, 2015). It is an important cereal crop that has assumed the status of a cash/food crop. Maize plays a predominant role in the farming systems and diets of millions of Nigerians. It is a very versatile crop since it is used for domestic consumption in addition to its industrial use by flour mills, breweries, confectioneries and animal feed manufacturers. This crop can be grown all year round provided there is available water. Consequently, increasing maize yields and its cultivation, particularly in high production potential areas of the country like Oyo State, can push a second maize green revolution. Maize is always preferred over any other crop including cassava because most of the world's civilizations developed around grains rather than tuber crops (Fakorede, 2001).

Despite the development and introduction of maize production technologies such as improved seed varieties, fertilizers, pesticides, herbicides, planters and irrigational systems by the existing research institutes such as the International Institute of Tropical Agriculture (IITA), National Cereal and Research Institute (NCRI) to increase the maize productivity level in Nigeria, maize average yield is still found to be very low (1/3 tons/ha) compared to its potential yield (Babatunde et al., 2008). To advance in the understanding of the economic impact and determinants of the adoption of the maize production technology package, this study explores the impact on net revenue from maize production. The following research questions drive this study:

i) What is the level of awareness of maize production technologies among farmers in the study area?

ii) What are the determinants of the adoption of the maize production technologies?

iii) What is the economic impact of the adoption of the maize production technologies?

Based on the literature review and new statistical evidence, this study attempts to analyse maize, required technologies to realisemajor productivity gains, and desirable government policies. More specifically, the study has the following objectives:

(i) to analyse the awareness level of maize production technologies among farmers in the study area;

(ii) to determine factors affecting the adoption of maize production technologies, and

(iii) to estimate the economic impact of the adoption of improved maize production technologies on net revenue from maize production in Oyo State, Nigeria.

Materials and Methods

Study area

The study was carried out in Oyo State of Nigeria which is one of the major maize producing states in Nigeria. The state is bounded in the north by Kwara State, in the east by Osun State and in the south by Ogun State. Oyo State is located in the south-western part of Nigeria. It is located between latitudes $7^0 3^1$and $9^0 12^1$ north of the equator and longitudes $2^0 47^1$ and $4^0 23^1$east of the meridian. The temperature is$27^0 C$. The state produces an average of 171,666.67 tonnes of maize per cropping season (FAO, 2006) which is about 50% of maize in the south-western part of Nigeria. The Oyo State Agricultural Development Programme (OYSADEP) divided the state into 4 agricultural zones, namely: Ibadan, Saki, Oyo and Ogbomosho zones. These zones were further divided into agricultural blocks and each block contained different cells.

Sampling procedure and sampling size

A multistage sampling procedure was adopted for this study. The first stage was the purposive selection of the Ibadan/Ibarapa Agricultural Development Programme zone based on the intensity of maize production by farm households in the area. The second stage was also the purposive selection of two (2) blocks from the Agricultural Development Programme zone based on the high concentration of maize farmers in the area. The third stage was the simple random sampling of two (2) cells from the selected blocks. The fourth stage was the simple random selection of three (3) communities from each of the two (2) cells and the fifth and last stage was the random selection of ten (10) maize producers from each community based on the list of the registered farmers obtained from the OYSADEP to arrive at a total number of 120 respondents.

Types and method of data collection

The study was a cross-sectional survey of 120 maize farmers in the study area. Primary data were collected with the aid of a structured questionnaire. Secondary sources of information such as journals, internet, literature and textbooks related to this topic were also consulted to complement the primary data.

Analytical techniques

Descriptive analysis

Descriptive statistics such as the frequency table and percentage were used to carry out the socio-economic analysis of maize farmers and to analyse the various types of maize production technologies used by farmers in the study area.

Adoption index

The adoption of the number of improved practices is usually measured by an adoption score (number of improved practices used) or by an adoption quotient (number of improved practices used over a total number of recommended practices). This study computed the adoption index to find out to what extent a farmer adopted a whole set of maize production technologies following Tadesse (2008), as shown in equation 1:

$$A.I_{=}\frac{1}{N_p}\sum_i^n\left[\frac{PSAi}{PR}+\frac{SRAi}{SR}+\frac{FAi}{FR}+\frac{HBAi}{HBR}+\frac{SDAi}{SDR}+\frac{DSAi}{SDR}+\frac{DSUi}{DPR}+\frac{MPUi}{MPR}\right] \quad (1)$$

where i = 1, 2, 3 ----------n,

$A.I$ = Adoption index of the i^{th} farmer;

N_p = Number of practices;

PSA_i = Plant spacing used by the i^{th} farmer (cm);

PR = Plant spacing recommended for the crop (cm);

SRA_i = Seeding rate used by the i^{th} farmer (kg/ha);

SR = Seed rate per hectare (kg/ha);

FA_i= Amount of fertiliser applied per hectare of area cultivated using improved maize varieties (kg/ha);

FR = Amount of fertiliser recommended for application per unit ofarea for improved maize production (kg/ha);

SDA_i = Seed dressing used by the i^{th} farmer (g/kg of seed);

SDR =Seed dressing recommended for the crop (g/kg of seed);

HBA_i = Amount of herbicide applied per hectare of area cultivated using improved maize varieties (litre/ha);

HBR_i = Amount of herbicide recommended per hectare of area cultivated

using improved maize varieties (litre/ha);

DSA=Date of sowing for the i^{th} farmer (months);

DSR_i = Date of sowing recommended for the improved maize (months);

DSU_i= Depth of sowing used by the i^{th} farmer (cm);

DPR =Depth of sowing recommended for the crop (cm);

MPU_i= Maturity periods used for harvesting maize by the i^{th} farmer (days), and

MPR = Maturity periods used for harvesting the crop (days).

The adoption index ranges from 0 to 1 depending upon the farmer's degree of the technology adoption. The overall adoption indices of all the farmerswere categorised into four distinct categories (non-adopters, low adopters, medium adopters and high adopters) following Maiangwa et al. (2007). The adoption score 0 point implies the non-adoption of the improved maize production package. If the index is above the value of 1, it indicates the farmers used some of the practices above the recommended rate.

Budgetary analytical technique

This was used to determine the economic benefits (profitability) of the adoption of maize production technologies among the adopters and non-adopters in the study area. While the gross margin could be regarded as the difference between the annual total revenue for each respondent and the variable costs directly associated with them, profitability is a measure of the level of performance using the available resources.

The gross margin is calculated as in equation 2:

$$GM_i = TR_i - VC_i \quad (2)$$

where:

GM_i = Gross margin for the i^{th}maize farmer;

TR_i = Total revenue for the i^{th}maize farmer;

VC_i = Variable cost for the i^{th}maize farmer, and

i = 1, 2, 3…i^{th} farmer.

Total revenue for the i^{th}maize farmer represents the product of quantity of output of maize and the unit price of output for each respondent. Variable cost for the i^{th}maize farmer represents all the expenses for growing maize. This includes: cost of improved maize seeds, cost of fertilizers, cost of mechanisation, cost of labour, and cost of herbicides.

Profitability was measured using the returns on investment (ROI) estimated by dividing the revenue by the total cost. The total cost was estimated by adding the

total variable cost to the total fixed cost incurred during production.

Regression model

We used the ordinary least square multiple regression analysis to determine the determinants of the adoption of maize production technologies in the study area (See equation 3):

$$y_i = \beta_o + X_i\beta_i + ---------+u_i \quad (3)$$

where:

y_i is the adoption of maize production technologies measured by the number of technologies used by the respondents in the last one year, i = the number of individual respondents 1, 2, 3; $\beta_0$is the constant term, $X_i$is the vector of independent variables, β_i is the vector of unknown coefficients, u_i is an error term.

The independent variables are defined as follows:

X_1= Sex (1 = male, 0 = female);

X_2= Age (years);

X_3 = Household size (number of household members);

X_4 = Farming experience (years);

X_5= Farm size (hectares);

X_6 = Years of formal education (years);

X_7 = Awareness level (index), and

X_8 = Extension visits (numbers of contacts with extension agents in the past one year).

Results and Discussion

Awareness level of improved maize production technologies

The results in Table 1 reveal that the majority of maize farmers were both aware and tried the improved maize seed varieties (69.2%) and organic fertiliser (83.3%), but never adopted any of them.

Table 1. The awareness level of improved maize production technologies.

Technologies	Not aware	Aware but never tried	Tried but not yet adopted	Adopted
Improved seeds	2(1.7%)	12(10%)	83(69.2%)	23(19.2%)
Organic fertiliser	1(0.8%)	1(0.8%)	100(83.3%)	18(15.00%)
Inorganic fertiliser	1(0.8%)	6(5%)	14(11.7%)	99(82.5%)
Knapsack sprayer	0(0.0%)	6(5.0%)	24(20.0%)	90(75.0%)
Pesticide	3(2.5%)	3(2.5%)	25(20.8%)	99(82.5%)
Improved pest scaring device	1(0.8%)	61(50.8)	3(2.5%)	55(45.8%)
Seed planter	40(33.3%)	75(62.5%)	4(3.3%)	0(0.0%)
Tractor	1(0.8%)	33(27.5%)	75(62.5%)	11(9.2%)
Grain harvester	39(32.5%)	78(65%)	3(2.5%)	0(0.0%)

Note: The value in parenthesis represents the percentage distribution.
Source: Data from field survey, 2016.

The most common maize production technologies farmers adopted in the study area were: inorganic fertilisers (82.5%), use of pesticide (82.5%), and use of knapsack sprayer (75.0). The least adopted technologies were: seed planter (0%), grain harvester (0%), tractor (9.2%) and improved pest scaring devices (45.8%).

Distribution of maize farmers by adoption index of production technologies

The results of categorisation of maize farmers on the adoption of improved maize technologies in the study area presented in Table 2 show that 12.5% of the sampled farmers lay in the low adopters' category, 3.33% in the non-adopter category, 76.7% were medium adopters and only 7.5% were high adopters. This implies that the overall adoption of the maize technology package was lower than the rate recommended by the producers and extension agents. Most of the farmers in the study area used the maize production technology packages below the recommended rates.

Table 2. Distribution of maize farmers by adoption index of production technologies.

Adoption categories	Adoption index	Frequency	Percentage
Non-adopters	0.00–0.009	4	3.3
Low adopters	0.01–0.33	15	12.5
Medium adopters	0.34–0.66	92	76.7
High adopters	0.67–1.00	9	7.5

Source: Data from field survey, 2016.

Economic impact of adoption of improved maize production technologies

The estimated economic impacts of the adoption of improved maize production technologies in the study presented in Table 3 show thatmaize farmers who are non-adopters of the production technology in the study areahad an average total variable cost of production of ₦63,271.06/ha. Out of this amount, the cost of herbicides alone constituted about 43.16% (₦27, 309.33) followed by the cost of fertilisers, ₦24,405.33 (38.57%); and labour cost ₦5,812.65 (9.19%). Other costs incurred by non-adopter farmers were the cost of seeds (₦5,206.25) and the cost of pesticides (₦537.50). Contrary to expectation, for maize farmers who adopted maize production technologies, the total variable cost of production was found to be ₦55,412.57/ha. Out of this amount, the cost of fertilisers alone constituted about 48.20% (₦26,710.67), followed by the cost of herbicides and chemicals ₦16,370.50 (29.55%) and labour cost took about 13.61% (₦7,538.73). Hence, it

was clearly shown that maize farmers who adopted improved maize production technologies had higher profit than the non-adopters and in terms of the performance using the available resources, production of maize using the improved maize production technologies was more profitable. The results in Table 3 show that for every one naira invested by the non-adopters, only ₦6.00 was made in returns against ₦7.64 by the adopters. This implies that the adoption of maize technology in the study area leads to more profitability in maize production.

Table 3. Analysis of the cost and returns of maize production of adopters and non-adopters per hectare per annum.

Costs	Non-adopters		Adopters	
	Amount (₦)	% in TVC	Amount (₦)	% in TVC
		Variable cost		
Cost of fertilisers	24,405.33	38.57	26,710.67	48.20
Cost of pesticides	537.50	0.85	508.30	0.91
Cost of seeds	5,206.25	8.23	4,284.37	7.73
Cost of labour	5,812.65	9.19	7,538.73	13.61
Cost of herbicides	27,309.33	43.16	16,370.50	29.55
TVC	63,271.06		55,412.57	
TFC	11,582.50		10,580.20	
Revenue (₦)	449,280.00		504,360.00	
GM (₦)	386,008.94		448,947.43	
Net profit (₦)	374,426.44		438,367.23	
ROI	6.00		7.64	

Note: TVC is the total variable cost, TFC is the total fixed cost, GM is the gross margin and ROI is the returns on investment which is a measure of profitability.
Source: Data from field survey, 2016.

Determinants of adoption of improved maize production technologies

The literature on agricultural technology adoption is vast (Rogers, 2003; Sunding and Zilberman, 2001; Feder and Umali, 1993) and somewhat difficult to summarise (Uaiene, 2011; Muzari et al., 2012; Ochienno, 2014). Our empirical results presented in Table 4 show that sex was a positive and significant ($P<0.01$) variable that influenced the adoption of maize technologies in the study area. This implies that the adoption of improved maize production technologies was gender sensitive. Similarly, farming experience had a positive influence on the adoption of improved maize production technologies at the 5% level of significance. This implies that as maize farmers increased their adoption level they advanced in farming experience. A more experienced farmer may have a lower level of uncertainty about the innovation performance and also be able to evaluate the advantage of the technology being considered. The variable 'Years of education' was also seen to have a positive and significant ($P < 0.01$) influence on the

adoption of maize technologies in the study area. This implies that the more educated a farmer was, the more likely to adopt any innovation. The education level of a farmer increased his/her ability to obtain, process and use the information relevant to the adoption of a new technology (Mignouna et al., 2011; Lavison, 2013; Namara et al., 2003). The results further show that extension visits and awareness of the production technologies had a positive and significant (both at $P < 0.01$) influence on the maize production adoption in the study area. This implies that the more extension visits/contact and awareness information farmers received, the more likely they adopted the technology. This is in line other past studies such as Nguluu et al. (1996) and Genius et al. (2014) who respectively found that acquisition of information about a new technology determines the adoption of technology and access to extension services helps to spread information about a new agricultural technology and hence its adoption.

Table 4. Determinants of adoption of maize production technologies.

Variables	Coefficients	Standard error	t-Stat
Constant	0.36520***	0.1150905	3.1731670
Sex	0.44220***	0.1257208	3.5173348
Age	0.000503	0.0016977	0.2961985
Household size	0.0090656	0.0070366	1.2883498
Farming exp.	0.00564**	0.0023582	2.3919360
Farm size	0.0236697	0.0160067	1.4787373
Years of education	0.04858***	0.0035028	13.8705197
Awareness index	0.15267***	0.0328187	4.6520475
Extension visits	0.07233***	0.0246093	2.9392017
F-statistics	35.8271		
p-value	0.0010		
R-squared	0.6552		
Observation	120		

Note: **, *** are the 5% and 1% significance levels respectively.
Source: Computed from data collected from field survey, 2016.

Conclusion

This study reveals that the majority (76.7%) of maize farmers in Oyo State were medium adopters of improved maize production technologies. The adoption of maize production technologies in the state was also found to be attributable to multiple factors like the sex of the farmers, farming experience, years of education, extension visits, and awareness level of farmers about the technologies. The adoption of maize production technology was more profitable in the study area. While on every naira invested in maize production, the adopters were able to make ₦7.64 in return, the non-adopters made ₦6.00. There is a need for an increase in the awareness level of maize production technologies through the extension agents

and social networks if the level of adoption of maize technologies is to be increased in the study area.

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EKONOMSKI UTICAJ I DETERMINANTE USVAJANJA POBOLJŠANIH TEHNOLOGIJA PROIZVODNJE KUKURUZA

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R e z i m e

Poteškoće oko toga koju proizvodnu tehnologiju usvojiti, i mere do koje bi trebalo poboljšati poljoprivredne operacije za postizanje optimalne ekonomske koristi ostaju neutvrđene. Ovim istraživanjem se analizira ekonomika i determinante usvajanja paketa poboljšanih tehnologija proizvodnje kukuruza (*Zea mays*) u Državi Ojo u Nigeriji. Korišćena je višestepena procedura uzorkovanja kako bi se odabralo sto dvadeset poljoprivrednih proizvođača kukuruza za istraživanje u 2016. godini. Podaci za istraživanje su prikupljeni korišćenjem strukturiranog upitnika, i analizirani su uz pomoć deskriptivne statistike i indeksa usvajanja (engl. *adoption index*), regresione analize i standardnih oblika kalkulacija za pojedinačne linije proizvodnje. Rezultati regresione analize su pokazali da promenljive kao što su pol, iskustvo bavljenja poljoprivredom, godine obrazovanja, posete savetodavaca, i nivo svesti o tehnologijama imaju značajnog i pozitivnog uticaja na usvajanje poboljšanih tehnologija za kukuruz u ispitivanom području. Rezultati analize standardnih oblika kalkulacija za pojedinačne linije proizvodnje su pokazali da su poljoprivrednici koji su usvojili poboljšane tehnologije proizvodnje kukuruza zaradili 438.367,23 naira u odnosu na profit od 374.426,44 naira po hektaru kukuruza koji su ostvarili poljoprivrednici koji nisu usvojili poboljšane tehnologije za proizvodnju kukuruza tokom godine istraživanja. Rezultati su dalje pokazali da na svaku nairu uloženu u proizvodnju kukuruza, poljoprivredni proizvoiđači koji su usvojili poboljšane tehnologije zaradili su 7,64 naira zauzvrat u poređenju sa prihodom od 6,00 naira koji su ostvarili poljoprivredni proizvođači koji nisu usvojili poboljšane tehnologije. Postoji potreba za povećanjem svesti o tehnologijama proizvodnje kukuruza među poljoprivrednim proizvođačima, uz pomoć savetodavaca i društvenih mreža, kako bi se povećao stepen usvajanja tehnologija za kukuruz.

Ključne reči: indeks usvajanja, ekonomska analiza, proizvodnja kukuruza, poljoprivredni proizvođači kukuruza.

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The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

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The examples of listing references are the following:

Periodicals

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Book chapter

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R. M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Proceedings
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Thesis
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Report
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Web site
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

Summary
The summary in Serbian is given at the end of the paper and should comprise 100 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

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Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

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All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

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Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature

The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

Formulae

All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

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Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod
Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode
Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

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U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

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U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

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Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

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Primeri navođenja referenci su sledeći:

Periodičan časopis
Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj
Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt
Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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Tabele
Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije
Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice
U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

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Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule
Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation“. Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

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63

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