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VALORIZATION OF NETTLE TEA INDUSTRY BY-PRODUCTS THROUGH INNOVATIVE GREEN EXTRACTION AND FERMENTATION FOR FUNCTIONAL FOOD APPLICATIONS

Mihajlo BOGDANOVIĆ^{1*}, Jovana GRBIĆ², Dragana MLADENOVIĆ², Ana ŽUGIĆ³, Vanja TADIĆ³,
Predrag PETROVIĆ², Aleksandra DJUKIĆ-VUKOVIĆ¹

¹Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11120 Belgrade,
Serbia

²Innovation Center of Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4,
11120 Belgrade, Serbia

³Department for Pharmaceutical Research and Development, Institute for Medicinal Plant Research
“Dr Josif Pančić”, Tadeuša Košćuška 1, 11000
Belgrade, Serbia

Contact: mbogdanovic@tmf.bg.ac.rs

Different food industry sectors generate substantial amounts of waste, posing a serious problem if not properly managed. The herbal tea industry by-products that remain after drying and cutting are rich in bioactive compounds such as polyphenols, flavonoids, phenolic acids, etc. however, they are underutilized. Approximately 10–40% (depending on plant species) of the total processed raw material in the herbal filter tea production is by-products in the form of pulvis. The common nettle (*Urtica dioica* L.) is an important part of human diet and it is commonly used for treating prostatic hyperplasia, hyperglycemia, allergies, anemia, etc., because of its diverse chemical profile. Since nettle is rich in polyphenols including flavonoids and phenolic acids, vitamins, minerals, etc. it is expected that it can be a good substrate for biotransformation by lactic acid bacteria. The optimized processing, including extraction and biotransformation, can convert these by-products into valuable sources of biologically active compounds for functional food applications. This study compares innovative strategies for the valorization of nettle by-products in accordance to the circular bioeconomy principles. Green techniques, including ultrasound-assisted and non-thermal plasma-assisted extraction were employed for the efficient recovery of bioactive compounds from nettle by-products.

Studied treatments and solvents (water and water/ethanol mixtures) were compared in order to examine their selectivity towards the antioxidant compounds which are suitable substrates for biotransformations. Extracts were used as substrates for the growth of *Ligilactibacillus salivarius* ATCC 11741, lactic acid bacteria strain with probiotic potential. Extracts were first lyophilized and later reconstituted with water, to obtain water-based media for *L. salivarius* growth. The antioxidant activity assays, ABTS, DPPH and total phenolic content as well as viability of *L. salivarius* were assessed. Further, biotransformation of aqueous extracts by fermentation with *L. salivarius* was confirmed by HPLC analysis. Fermentation improved the quality of the extract by increasing the concentration of phenolic compounds, namely, caffeic acid (44%), chlorogenic acid (35%) and caffeic acid derivatives (44%), while maintaining a high growth rate of tested microorganism. DPPH assay showed high activity of 234 trolox eq/g dm in non-thermal plasma extracted fermented extracts. Although non-thermal plasma is used for its sterilization and antimicrobial properties in some cases, *L. salivarius* showed high viability in both non-thermal plasma and ultrasound treated extracts. The fermentation with *L. salivarius* was directly associated with an increase in the concentration of biologically active compounds, which opens new opportunities for biotransformation of extracts, potentially also with other lactic acid bacteria to obtain new specific fermentation products.

The fermented aqueous extracts showed significant potential as symbiotics with antioxidant and postbiotic properties. The stability of fermented extracts should be further examined in order to propose best route for their exploitation, but increased biological activity suggests that their applications could span from functional food to dietary supplements and cosmetic ingredients.

Key words: common nettle, non-thermal plasma, ultrasound extraction, *Ligilactibacillus salivarius*, biotransformation, green extraction.

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VALORIZACIJA SPOREDNIH PROIZVODA INDUSTRIJE ČAJA KOPRIVE KROZ INOVATIVNU ZELENU EKSTRAKCIJU I FERMENTACIJU ZA PRIMENU U FUNKCIONALHOJ HRANI

Mihajlo BOGDANOVIĆ^{1*}, Jovana GRBIĆ², Dragana MLADENOVIĆ², Ana ŽUGIĆ³, Vanja TADIĆ³,
Predrag PETROVIĆ², Aleksandra DJUKIĆ-VUKOVIĆ¹

¹ Tehnološko-metalurški fakultet Univerziteta u Beogradu, Karnegijeva 4, 11120 Beograd, Srbija

² Inovacioni centar Tehnološko-metalurškog fakulteta Univerziteta u Beogradu, Karnegijeva 4, 11120
Beograd, Srbija

³ Department for Pharmaceutical Research and Development, Institute for Medicinal Plant Research
“Dr Josif Pančić”, Tadeuša Koščuška 1, 11000
Belgrade, Serbia

Kontakt: mbogdanovic@tmf.bg.ac.rs

Različiti sektori prehrambene industrije stvaraju značajnu količinu otpada, što predstavlja ozbiljan problem ako se njime ne upravlja na pravi način. Sporedni proizvodi industrije biljnog čaja koji ostaju nakon sušenja i sečenja bogati su bioaktivnim jedinjenjima kao što su polifenoli, flavonoidi, fenolne kiseline itd., međutim, nedovoljno iskorišćeni. Otprilike 10–40% (u zavisnosti od biljne vrste) ukupne prerađene sirovine u proizvodnji biljnog filter čaja su sporedni proizvodi u obliku pulvisa. Kopriva (*Urtica dioica*) je važan deo ljudske ishrane i najčešće se koristi za lečenje hiperplazije prostate, hiperglikemije, alergija, anemije itd., zbog svog raznovrsnog hemijskog profila. Pošto je kopriva bogata polifenolima uključujući flavonoide i fenolne kiseline, vitamine, minerale itd., očekuje se da može biti dobar supstrat za biotransformaciju bakterija mlečne kiseline. Optimizovana prerada, uključujući ekstrakciju i biotransformaciju, može pretvoriti ove nusproizvode u vredne izvore biološki aktivnih jedinjenja za funkcionalnu primenu u hrani. Ova studija upoređuje inovativne metode valorizacije nusproizvoda koprive u skladu sa principima kružne bioekonomije. Zelene tehnike, uključujući ultrazvučnu i ekstrakciju uz pomoć netermalne plazme, korišćene su za efikasan oporavak bioaktivnih jedinjenja iz nusproizvoda koprive.

Upoređeni su i proučavani tretmani i rastvarači (mešavine voda i voda/etanol) kako bi se ispitala njihova selektivnost prema antioksidativnim jedinjenjima koja su pogodni kao supstrati za biotransformacije. Ekstrakti su korišćeni kao supstrati za rast *Ligilactibacillus salivarius* ATCC 11741, soja bakterija mlečne kiseline sa probiotičkim potencijalom. Ekstrakti su liofilizovani i kasnije rekonstituisani vodom, da bi se dobio medijum na bazi vode za rast *L. salivarius*. Procenjivane su antioksidativne aktivnosti, ABTS, DPPH i ukupni fenolni sadržaj, kao i rast *L. salivarius*. Dalje, biotransformacija vodenih ekstrakata fermentacijom sa *L. salivarius* je potvrđena HPLC analizom. Fermentacija je poboljšala kvalitet ekstrakta povećanjem koncentracije fenolnih jedinjenja, i to kofeinske kiseline (44%), hlorogenske kiseline (35%) i derivata kafeinske kiseline (44%), uz održavanje visoke stope rasta ispitivanog mikroorganizma. DPPH test je pokazao visoku aktivnost od 234 trolok ek/g dm u fermentisanim ekstraktima dobijenim primenom netermalne plazme. Iako se netermalna plazma u nekim slučajevima koristi u antimikrobne svrhe, *L. salivarius* je pokazao visoki rast i u ekstraktima dobijenim uz primenu netermalne plazme i u ekstraktima ekstrahovanim uz asistenciju ultrazvukom. Fermentacija sa *L. salivarius* bila je direktno povezana sa povećanjem koncentracije biološki aktivnih jedinjenja, što otvara nove mogućnosti za biotransformaciju ekstrakata, potencijalno i sa drugim bakterijama mlečne kiseline za dobijanje novih specifičnih proizvoda fermentacije.

Fermentisani vodeni ekstrakti su pokazali značajan potencijal kao simbiotici sa antioksidativnim i postbiotičkim svojstvima. Stabilnost fermentisanih ekstrakata treba dalje ispitati kako bi se predložio najbolji put za njihovu eksploataciju, ali povećana biološka aktivnost sugerise da bi njihova primena mogla da se proteže od funkcionalne hrane do dijetetskih suplemenata i kozmetičkih sastojaka.

Ključne reči: obična kopriva, netermalna plazma, ultrazvučna ekstrakcija *Ligilactibacillus salivarius*, biotransformacija, zelena ekstrakcija.

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