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INOPTEP 2025
and
XXXVII NATIONAL CONFERENCE
PROCESSING AND ENERGY
IN AGRICULTURE
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i
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CONTENT

THE STRUCTURE OF THE PROTEIN CONCENTRATE OBTAINED BY THE ULTRASONIC AND CHEMICAL DEPROTEINIZATION PROCESS OF THE SPINY CRAYFISH SHELL FROM THE DANUBE RIVER

Nevena HROMIŠ[1], Senka POPOVIĆ[1], Danijela ŠUPUT[1], Jovana PANTIĆ[1], Sandra BULUT[1], Zorica TOMIČIĆ[2], Ivana ČABARKAPA[2]
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The invasive species of river crayfish Faxonius limosus, which has a significant negative impact on the biodiversity of rivers in which it overproliferates, endangering other species of crayfish and fish, has been detected in Serbia throughout the entire course of the Danube, as well as the Sava, Tisa, Tamiš and Velika Morava rivers. One possible approach to controlling this problem is to catch individuals of the spiny cheek crayfish in order to use their meat for human consumption. When the crayfish meat is removed, a large amount of shell remains, which can be a source of highly valuable components, such as chitin, chitosan, protein, astaxanthin and others.

The usual and most commonly used method of extracting protein from crayfish shells is the use of strong base baths at elevated temperatures. This method has proven to be effective, but its main drawback is the use of a significant amount of strong base. As a green alternative to this extraction method, extraction using ultrasound in water, at room temperature, has been proposed.

In this paper, the structure of protein concentrate isolated by chemical and ultrasonic deproteinization processes is compared. The analysis of the amino acid composition indicated a slightly higher total content of amino acids in the case of the classic chemical method of isolation, whereby this difference mainly comes from the content of essential amino acids. Non-polar amino acids dominate over polar amino acids in both concentrates (three times higher), while the ratio of hydrophilic and hydrophobic amino acids is different in these two concentrates. Namely, the chemically obtained concentrate has a uniform ratio of hydrophilic and hydrophobic amino acids, while in the case of the ultrasonic concentrate, this ratio is in favor of the hydrophilic ones. Of all amino acids, the highest content was found for aspartic and glutamic acids, followed by leucine and phenylalanine in the chemical concentrate, and leucine and lysine in the ultrasonic concentrate.

FTIR analysis of the spectrum of protein concentrates revealed peaks typical for proteins, amide I (1700–1600 cm^{-1}), amide II (1600–1500 cm^{-1}) and amide III (1300–1200 cm^{-1}). By analyzing the shape and position of the amide peaks, as well as by deconvolution of the amide I peak, an assessment of the secondary structure of the protein in the protein concentrate was obtained. In both concentrates, the most abundant conformation is random. In the case of the chemical concentrate, the β-sheet follows and then the α-helix, while in the case of the UV concentrate, these conformations are equally represented. The more well-preserved and ordered structure of the UV concentrate may be a consequence of the lower temperature and the absence of chemicals during extraction.

The obtained protein concentrates can represent an additional valorization of the shell of the river crayfish and have a significant application for animal feed or for obtaining biodegradable packaging, or edible casings for food or feed products.

Key words: *protein concentrate, river crayfish, FTIR, conformation, amino-acid content*

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STRUKTURA PROTEINSKOG KONCENTRATA DOBIJENOG ULTRAZVUČNIM I HEMIJSKIM POSTUPKOM DEPROTEINIZACIJE LJUŠTURE BODLJOBRADOG REČNOG RAKA IZ REKE DUNAV

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Invazivna vrsta rečnog raka Faxonius limosus, koja umnogome negativno utiče na biodiverzitet reka u kojima se prenamnoži ugrožavajući ostale vrste rakova, ali i riba, detektovan je u Srbiji već u celom toku reke Dunav, ali i Save, Tise, Tamiša i Velike Morave. Jedan od mogućih pristupa da se ovaj problem stavi pod kontrolu je i izlovljavanje jedinki bodljobradog raka radi upotrebe njegovog mesa za ishranu ljudi. Kada se meso raka ukloni, ostaje velika količina ljušture koja može biti izvor visokovrednih komponenata, kao što su hitin, hitozan, protein, astaksantin i drugo.

Klasičan i najčešće upotrebljavan način ekstrakcije proteina iz ljušture rečnog raka je upotreba kupki jake baze, uz povišenu temperaturu. Ova metoda se pokazala efikasnom, ali joj je glavni nedostatak upotreba značajne količine jake baze. Kao zelena alternative ovom načinu ekstrakcije, ponuđena je ekstrakcija primenom ultrazvuka u vodi, pri sobnoj temperature.

U ovom radu je poređena struktura proteinskog koncentrata izolovanog hemijskim i ultrazvučnim procesom deproteinizacije. Analiza aminokiselinskog sastava je ukazala na nešto veći ukupni sadržaj aminokiselina kod klasičnog hemijskog načina izolacije, pri čemu ova razlika pretežno potiče od sadržaja esencijalnih aminokiselina. Nepolarne aminokiseline dominiraju nad polarnim kod oba koncentrata (trostruko više), dok je odnos hidrofilnih i hidrofobnih različit kod ova dva koncentrata. Naime, hemijski dobijen koncentrat ima ujednačen odnos hidrofilnih i hidrofobnih aminokiselina, dok je kod ultrazvučnog koncentrata, ovaj odnos u korist hidrofilnih. Od svih amino kiselina, najveći sadržaj je asparaginske i glutaminske kiseline, a zatim slede leucin i fenilalanin kod hemijskog izolata, a leucin i lizin kod ultrazvučnog koncentrata.

FTIR analizom spektara proteinskih koncentrata, detektovani su pikovi svojstveni za proteine, amid I (1700–1600 cm^{-1}), amid II (1600–1500 cm^{-1}) i amid III (1300–1200 cm^{-1}). Analizom oblika i položaja amidnih pikova, kao i dekonvolucijom amid I pika, dobijena je procena sekundarne strukture proteina u proteinskom koncentratu. Kod oba koncentrata, najzastupljenija konformacija je “random”. Za njom, kod hemijskog koncentrata sledi “β-sheet”, pa “α-heliks”, dok su kod UV koncentrata ove konformacije podjednako zastupljene. Očuvanija uređenija struktura kod UV koncentrata može biti posledica niže temperature i odsustva hemikalija prilikom ekstrakcije.

Dobijeni proteinski koncentrati mogu predstavljati dodatnu valorizaciju ljušture rečnog raka i imati značajnu primenu za hranu za životinje ili za dobijanje biorazgradive ambalaže, ili jestivih omotača za prehrambene proizvode.

***Ključne reči**: proteinski koncentrat, rečni rak, FTIR, konformacija, aminokiselinski sastav*

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POTENTIAL APPLICATION OF CHITOSAN-BASED BIOPOLYMER COATINGS ON FERMENTED DRY SAUSAGES: VARIOUS APPLICATION TECHNIQUES AND THEIR IMPACT ON PRODUCT QUALITY

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Chitin is the second most abundant organic resource on Earth, found in plants, marine invertebrates, insects, fungal cell walls, and certain microorganisms. Despite its widespread availability, chitin is commercially produced almost exclusively from crustacean shells, primarily due to the large quantities of exoskeleton waste generated by the seafood industry. To evaluate the potential of chitosan-based coatings for fermented sausages, commercially available chitosan derived from crustacean shells was used.

Chitosan-based biopolymer coatings were applied to 2 mm thick slices of fermented dry sausage using two techniques: spraying and immersion. A total of six different treatments were performed, differing in drying conditions after coating application. The spray technique involved three successive applications per side, followed by drying in either a ventilated oven (35°C) or a climate chamber (25°C, 15% relative humidity). The immersion technique consisted of three consecutive dipping and draining cycles, followed by drying under the same conditions. An untreated sample served as a control. To assess the effectiveness of the coatings samples were analysed through instrumental color measurements and sensory evaluation of aroma, taste, and overall acceptability.

Instrumental color analysis showed statistically significant ($P < 0.05$) changes in coated samples compared to the control. Immersion-treated samples had lightness values closer to the control, while spray-treated samples exhibited higher lightness and greater yellow color reduction. Sensory evaluation indicated that all samples, except S_3 (spray technique, drying in a climate chamber), were acceptable, with U_1 (immersion technique, drying in a ventilated oven/10 minutes) showing no significant difference from the control in aroma, taste, and overall acceptability. These findings suggest that chitosan-based coatings have potential for application on fermented dry sausages, with the U_1 immersion technique being the optimal treatment.

Key words: *chitosan, biopolymer coatings, fermented dry sausages, color analysis, sensory evaluation.*

Acknowledgments: This research was financially supported by the Science Fund of the Republic of Serbia, PROJECT NUMBER 7471, Reducing the negative impact of the invasive crayfish *Faxonitis limosus* in the Danube through the smart exploitation of their meat and shells - DANUBEcare.

POTENCIJAL PRIMENE HITOZANSKIH BIOPOLIMERNIH PREMAZA NA FERMENTISANE SUVE KOBASICE: UTICAJ RAZLIČITIH TEHNIKA NANOŠENJA NA KVALITET PROIZVODA

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Hitozan je drugi najzastupljeniji organski resurs na Zemlji, prisutan u biljkama, morskim beskičmenjacima, insektima, ćelijskim zidovima gljiva i određenim mikroorganizmima. Uprkos njegovoj širokoj dostupnosti, komercijalna proizvodnja hitozana gotovo isključivo potiče iz ljuštura rakova, prvenstveno zbog velikih količina egzoskeletnog otpada koji nastaje u industriji morskih plodova. U cilju ispitivanja potencijala hitozanskih premaza za fermentisane suve kobasice, korišćen je komercijalno dostupan hitozan dobijen iz ljuštura rakova.

Na tanko narezane listiće čajne kobasice, debljine 2 mm, nanošeni su hitozanski biopolimerni premazi korišćenjem dve tehnike: sprej tehnike i tehnike uranjanja. Ukupno je sprovedeno šest različitih tretmana, koji su se razlikovali prema uslovima sušenja nakon nanošenja premaza. Sprej tehnika obuhvatala je tri uzastopna nanošenja premaza sa obe strane uzorka, nakon čega je sledilo sušenje u sušnici sa cirkulacijom vazduha (35°C) ili u klima komori (25°C, 15% relativne vlažnosti). Tehnika uranjanja sastojala se od tri uzastopna ciklusa potapanja i ceđenja uzoraka, nakon čega je sledilo sušenje pod istim uslovima. Uzorak bez tretmana služio je kao kontrola. Efikasnost premaza procenjena je instrumentalnim merenjem boje i senzorskom evaluacijom arome, ukusa i ukupne prihvatljivosti.

Instrumentalna analiza boje pokazala je statistički značajne ($P < 0,05$) promene kod premazanih uzoraka u poređenju sa kontrolom. Uzorci tretirani uranjanjem imali su vrednosti svetloće boje bliže kontroli, dok su uzorci tretirani sprej tehnikom pokazali veću svetloću i izraženije smanjenje žute boje. Senzorska evaluacija pokazala je da su svi uzorci, osim S_3 (sprej tehnika, sušenje u klimatskoj komori), bili prihvatljivi, pri čemu U_1 (tehnika uranjanja, sušenje u sušnici sa cirkulacijom vazduha/10 minuta) nije pokazao značajnu razliku u odnosu na kontrolu u pogledu arome, ukusa i ukupne prihvatljivosti. Ovi rezultati sugerišu da hitozanski premazi imaju potencijal za primenu na fermentisanim suvim kobasicama, pri čemu se U_1 tehnika uranjanja pokazala kao optimalan tretman.

***Ključne reči*:** *hitozan, biopolimerni premazi, fermentisane suve kobasice, analiza boje, senzorska evaluacija*

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