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INOPTEP 2023**

and

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EXTRACTION OF HYDROXYMETHYLFURFURAL FROM HONEY USING AQUEOUS TWO-PHASE SYSTEMS

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Honey is a natural product that contains about 200 different compounds. It consists of carbohydrates, water, and other components, such as proteins (enzymes), organic acids, vitamins (especially vitamin B6, thiamin, niacin, riboflavin, and pantothenic acid), minerals, pigments, phenolic compounds, various volatile compounds, Maillard reaction products and caramelization products. 5-Hydroxymethylfurfural (HMF) is formed as an intermediate in the Maillard reaction and directly by dehydration of sugars in acidic conditions (caramelization) during thermal treatments of honey. In acidic conditions, HMF can be formed even at low temperatures, although its content increases dramatically with increasing temperature. Apart from the temperature, the amount of HMF formed also depends on the pH value, water activity, as well as concentration of divalent cations in the medium. *In vitro* toxicological studies have shown that HMF in high concentrations is cytotoxic, causing irritation of the eyes, upper respiratory tract, skin, and mucous membranes. *In vitro* experiments on animals have shown that exposure to high concentrations of HMF can cause skin cancer, kidney tumors, and increase the frequency of hepatocellular adenomas. In the middle 1950s, Albertsson proposed the use of aqueous two-phase systems as an alternative to traditional liquid-liquid extraction techniques, which usually require the use of volatile and toxic organic solvents. The aqueous two-phase system consists of two immiscible aqueous phases and is based on the combination of polymer-polymer, polymer-salt, and salt-salt. Although both substances are soluble in water, they separate into two coexisting phases above a certain concentration – with one phase rich in one and the other phase rich in the other component. Among them, the most studied class is ionic liquid-salt systems due to the high ability of salt ions to induce saltation of the ionic liquid and consequently create two aqueous phases.

The aim of this work was to develop and implement extraction procedures for HMF isolation from honey in order to ensure its safety status and to enable the further application of isolated HMF in various industry sectors. Bio-ionic liquids are seen as an ideal extractant for both purposes, and additionally, they can be reused lowering the costs of the analysis/process, as well as environmental pollution. By applying all tested aqueous two-phase systems based on choline ionic liquids (choline chloride ([Ch][Cl]), choline nicotinate ([Ch][Nic]), choline propionate ([Ch][Prop]) and choline butyrate ([Ch][But]) and inorganic salt K₃PO₄ for the extraction of HMF from honey, the extraction efficiency of more than 85% was achieved. Complete extraction ($EE_{\text{HMF}} = 100\%$) was achieved using the extraction system with [Ch][But], while the weakest ability to extract HMF exhibited the system with [Ch][Cl].

Key words: honey, HMF, aqueous two-phase systems

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EKSTRAKCIJA HIDROKSIMETILFURFURALA IZ MEDA PRIMENOM DVOFAZNIH VODENIH SISTEMA

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Med je prirodni proizvod koji sadrži oko 200 različitih jedinjenja. Sastoji se od ugljenih hidrata, vode i drugih komponenata, kao što su proteini (enzimi), organske kiseline, vitamini (posebno vitamin B6, tiamin, niacin, riboflavin i pantotenska kiselina), minerali, pigmenti, fenolna jedinjenja, najrazličitija isparljiva jedinjenja i proizvodi Maillard-ove reakcije i procesa karamelizacije. 5-Hidroksimetilfurfural (HMF) se formira kao intermedijer u Maillard-ovoj reakciji i direktno, dehidratacijom šećera u kiseloj sredini (karamelizacija) tokom termičkih tretmana meda. U kiselim uslovima, HMF se može formirati i pri niskim temperaturama, iako se njegov sadržaj drastično povećava sa temperaturom. Osim temperature, količina formiranog HMF zavisi i od pH vrednosti, aktivnosti vode, kao i od koncentracije dvovalentnih katjona u medijumu. *In vitro* toksikološke studije pokazale su da je HMF u velikim koncentracijama citotoksičan, izaziva iritaciju očiju, gornjeg respiratornog trakta, kože i sluzokože. *In vitro* eksperimenti na životinjama pokazali su da izloženost većim koncentracijama HMF može prouzrokovati karcinom kože, tumore na bubrezima, kao i da povećava učestalost hepatocelularnih adenoma. Sredinom pedesetih godina XX veka, Albertsson je predložio upotrebu dvofaznih vodenih sistema kao alternativu tradicionalnim tehnikama tečno-tečne ekstrakcije, koje uobičajeno iziskuju korišćenje isparljivih i toksičnih organskih rastvarača. Dvofazni vodeni sistem se sastoji od dve vodene faze koje se ne mešaju i zasnovane su na kombinaciji polimer-polimer, polimer-so i so-so. Iako su obe supstance rastvorljive u vodi, one se razdvajaju u dve koegzistirajuće faze iznad određene koncentracije – pri čemu je jedna faza bogata jednom, a druga faza drugom komponentom. Među njima, najproučavanija klasa su sistemi jonska tečnost-so zbog visoke sposobnosti jona soli da indukuju isoljavanje jonske tečnosti i konsekventno stvore dve vodene faze.

Cilj ovog rada je bio razvoj i implementacija ekstrakcionih postupaka za izolovanje HMF iz meda, kako bi se obezbedila njegova zdravstvena bezbednost i omogućila dalja primena izolovanog HMF u različitim granama industrije. Bio-jonske tečnosti se sagledavaju kao idealan ekstragens u svrhu obe namene, a dodatno, mogu se ponovo upotrebljavati (reciklirati), što snižava cenu analize/procesa, kao i zagađenje životne sredine. Primenom svih ispitanih dvofaznih vodenih sistema na bazi holinskih jonskih tečnosti (holin hlorid ([Ch][Cl]), holin nikotinat ([Ch][Nic]), holin propionat ([Ch][Prop]) i holin butirat ([Ch][But]) i neorganske soli K₃PO₄ za ekstrakciju HMF iz meda postignuta je efikasnost ekstrakcije veća od 85%. Potpuna ekstrakcija ($EE_{HMF} = 100\%$) postignuta je primenom ekstrakcionog sistema sa [Ch][But], dok je najslabiju sposobnost da ekstrahuje HMF ispoljio sistem sa [Ch][Cl].

Ključne reči: med, HMF, dvofazni vodeni sistemi

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