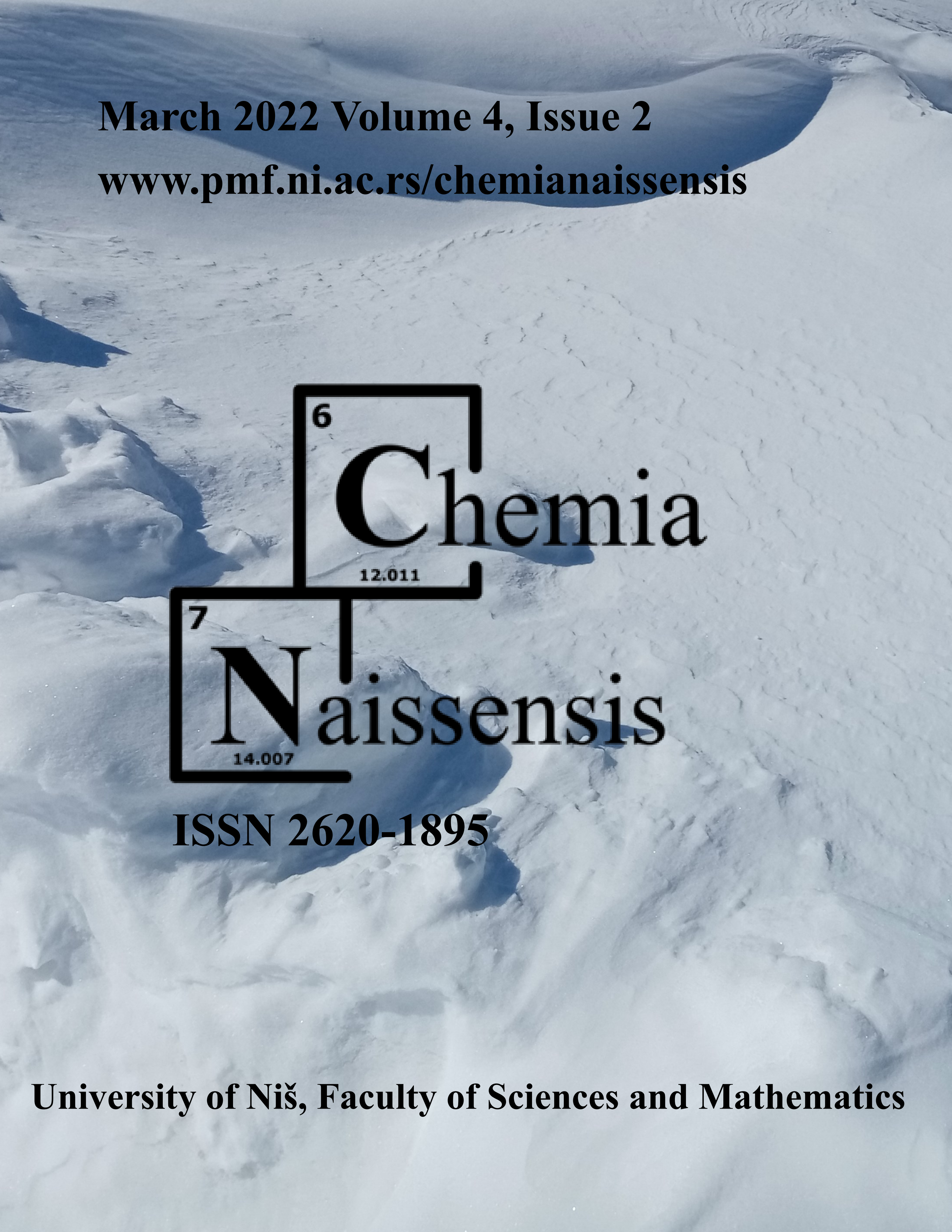

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Nobel prize laureates for chemistry in the period 2000-2021

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ABSTRACT

The Nobel Prize in Chemistry has a long history since 1901. There were significant changes and discoveries in chemistry in the previous period, especially intensified in the period 2000-2021. There was the development of new scientific disciplines in chemistry, some of which became completely independent. Also, scientific discoveries are increasingly multidisciplinary and the result of the work of large scientific teams. However, all this is not accompanied by an increase in the number of areas for which the Nobel Prize is awarded. The classification of scientific achievements into one of the six areas for which the Nobel Prize is awarded is increasingly tricky due to their complexity and multidisciplinarity. In the period 2000-2021, the largest number of Nobel Prizes have been awarded for research in the field of biochemistry (11), organic synthesis (4), development of new methods and materials (4), and others (3). All this indicates the dominance of research in biochemistry and life sciences and its independence, which is not adequately accompanied by changes in the structure and areas for which the Nobel Prizes are awarded. In the near future, the Nobel Committee should consider this issue and harmonize it with modern trends in science.

Keywords: Nobel Prizes in Chemistry, analysis

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Introduction

It was initially believed that biomolecules could only be formed in living organisms under the influence of the special life force *vis vitalis*. Such a belief caused the slow development of organic chemistry. In 1828, Friedrich Wöhler was the first to synthesize a biomolecule in the laboratory, urea. This moment was the beginning of modern organic chemistry. The discovery of the first enzyme-diastase in 1833 by Anselme Payen is considered the beginning of biochemistry. It is believed that in 1903, the German scientist Carl Neuberg gave science its current name, biochemistry. Since then, biochemistry has progressed rapidly, followed by the awarding of the Nobel Prize in Chemistry for discoveries in biochemistry.

Only three Nobel Prizes in Chemistry were awarded, which were related to discoveries in biochemistry in the first half of the 20th century (Kostic, 2010):

Buchner (1907) for the discovery of sugar fermentation without the presence of yeast cells;

Harden (1929) to investigate the fermentation of sugars and the enzymes involved;

Sumner, Northrop, and Stanley (1946) for the crystallization of enzymes and the isolation of enzymes and viruses in the pure state.

Numerous instrumental methods were developed, such as chromatography, X-ray diffraction, electron microscopy, nuclear magnetic resonance, isotope labeling, and others, which contributed to significant discoveries in biochemistry. Eight Nobel Prizes in Chemistry were awarded for discoveries in biochemistry (1970-1997).

Nobel prize in the period 2000-2021

The development of modern methods of analysis has enabled the development of biochemistry. Many molecules were analyzed in detail and their activity in the cells themselves at the molecular level. Complex biological systems, metabolic processes, chemical signaling, and neural function were analyzed.

Table 1 shows the winners of the Nobel Prize in Chemistry, discoveries related to biochemistry and life sciences, organic synthesis of new materials and methods.

Table 1. Nobel prize laureates for chemistry in the period 2000-2021 (www.nobelprize.org)

Year	Scientist	Country	Discovery	Field
2000	Alan J. Heeger	USA	discovery of plastics that conduct electricity	new materials and methods
	Alan G. MacDiarmid	USA		
	Shirakawa Hideki	Japan		
2001	William S. Knowles	USA	work on chirally catalyzed hydrogenation reactions	organic synthesis
	Noyori Ryoji	Japan		
	K. Barry Sharpless	USA		
2002	John B. Fenn	USA	development of techniques to identify and analyze proteins and other large molecules	biochemistry and life science
	Tanaka Koichi	Japan		
	Kurt Wüthrich	Switzerland		
2003	Peter Agre	USA	discoveries regarding water channels and ion channels in cells	biochemistry and life science
	Roderick MacKinnon	USA		
2004	Aaron Ciechanover	Israel	discovery of ubiquitin-mediated protein degradation	biochemistry and life science
	Avram Hershko	Israel		
	Irwin Rose	USA		
2005	Yves Chauvin	France	development of the metathesis method in organic synthesis	organic synthesis
	Robert H. Grubbs	USA		
	Richard R. Schrock	USA		
2006	Roger D. Kornberg	USA	work concerning the molecular basis of eukaryotic transcription	biochemistry and life science
2007	Gerhard Ertl	Germany	studies of chemical processes on solid surfaces	other
2008	Martin Chalfie	USA		
	Osamu Shimomura	USA		

	Roger Y. Tsien	USA	discovery and development of the green fluorescent protein, GFP	biochemistry and life science
2009	Venkatraman Ramakrishnan	USA	studies of the structure and function of the ribosome	biochemistry and life science
	Thomas Steitz	USA		
	Ada Yonath	Israel		
2010	Richard F. Heck	USA	development of techniques to synthesize complex carbon molecules	organic synthesis
	Negishi Ei-ichi	Japan		
	Suzuki Akira	Japan		
2011	Daniel Shechtman	Israel	discovery of quasicrystals	
2012	Brian K. Kobilka	USA	studies of G-protein-coupled receptors	biochemistry and life science
	Robert J. Lefkowitz	USA		
2013	Martin Karplus	Austria/USA	development of multiscale models for complex chemical systems	other
	Michael Levitt	UK/USA/Israel		
	Arieh Warshel	Israel/USA		
2014	Eric Betzig	USA	development of super-resolved fluorescence microscopy	new materials and methods
	Stefan W. Hell	Germany		
	William E. Moerner	USA		
2015	Tomas Lindahl	Sweden	mechanistic studies of DNA repair	biochemistry and life science
	Paul Modrich	USA		
	Aziz Sancar	Turkey/USA		
2016	Jean-Pierre Sauvage	France	design and synthesis of molecular machines	biochemistry and life science
	J. Fraser Stoddart	UK		
	Bernard Feringa	Netherlands		
2017	Jacques Dubochet	Switzerland		

	Joachim Frank	Germany/USA	development of cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution	new materials and methods
	Richard Henderson	UK		
2018	Frances Arnold	USA	first directed evolution of enzymes	biochemistry and life science
	George P. Smith	USA	development of phage display, a method in which a bacteriophage can be used to evolve new proteins	
	Gregory P. Winter	UK	work using the phage display method for the directed evolution of antibodies	
2019	John B. Goodenough	USA	development of lithium-ion batteries	new materials and methods
	M. Stanley Whittingham	UK/USA		
	Yoshino Akira	Japan		
2020	Emmanuelle Charpentier	France	development of a method for genome editing	biochemistry and life science
	Jennifer Doudna	USA		
2021	Benjamin List	Germany	development of asymmetric organocatalysis	organic synthesis
	David W.C. MacMillan	UK/USA		

The fact is that since the 1970s, more and more biochemists have won the Nobel Prize in Chemistry, and fewer and fewer have won the Nobel Prize in Physiology or Medicine. In genetics, some discoveries have received awards in physiology or medicine. In contrast, others have received an award for chemistry. Some laureates in chemistry could equally be awarded a prize for physiology or medicine (Lindsten & Ringertz, 2001).

The Nobel Foundation and the Royal Swedish Academy of Sciences gradually changed the character and function of the Nobel Prize in Chemistry. Eleven of the last 22 Nobel Prizes in Chemistry have been awarded for biochemistry and molecular biology achievements.

When writing his will, Alfred Nobel could not have imagined the incredibly rapid development of science in the coming period. Although he did not favor the sciences for which the Nobel Prize would be awarded, Nobel may have considered chemistry the most important. Chemistry was his profession, and chemistry brought him the wealth that enabled the formation of this foundation (Kauffman, 2001).

Are life sciences and chemistry two parts of the same discipline? If so, then the temporal characteristics of the Nobel Prize in Chemistry favoring the life sciences would reflect the reshaping of science. The interdisciplinary nature of much of today's research further disrupts all sharp divisions within the boundaries of rewards (Szell et al., 2018).

Ambiguity and flexibility in setting individual Nobel Prizes within various disciplines go back long. There is a historical record of inconsistent awarding prizes by Nobel Prize-winning bodies.

Are chemistry and biochemistry one discipline so that the achievements in both are appropriately rewarded with the same Nobel Prize, the Nobel Prize in Chemistry? If so, why are so many achievements in biochemistry in the first 60 years awarded the Nobel Prize in Physiology or Medicine?

Despite early points of contact, biochemistry has been constituted since 1920 as a new discipline and profession with specialized journals.

The differentiation of biochemistry from mainstream chemistry does not imply the disappearance of a fundamental aspect of chemistry in biochemistry but rather an evolution on the path of specialization of both disciplines with some points of contact. In the past, biochemistry used scientists and methods from chemistry to later develop its methodologies and its own research goals.

So, in the period 2000-2021, 11 Nobel Prizes have been awarded for research in biochemistry, with the Nobel Prize for 2018 being awarded to three scientists for three different types of research in the field of biochemistry.

In 2000-2021, four Nobel Prizes in Chemistry for Organic Synthesis were awarded.

Research in organic synthesis must continue to keep in step with the need to synthesize increasingly complex target molecules and the need to have synthetic methods characterized by greater ease and efficiency at our disposal. These fundamental exercises, coupled with the many avenues available for applying organic synthesis, will continue to foster creative works in this area throughout the 21st century.

The Nobel Prizes for the discovery of modern materials and instrumental methods were also awarded: discovery of plastics that conduct electricity, development of super-resolved fluorescence microscopy, development of cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution, development of lithium-ion batteries.

Other discoveries are from other fields of chemistry: studies of chemical processes on solid surfaces discovery of quasicrystals.

Despite the growing interdisciplinarity of research, the Nobel Prize maintains the traditional disciplinary categorization of science.

The interdisciplinary nature of the award is best represented at the Nobel Prize in Chemistry, which was awarded in 2017-the development of cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution. The physical technique was developed with the help of chemistry and was applied to study biomolecules. This indicates that the barriers in question should not hinder the development of science. However, it should be understood that the most revolutionary discoveries in the period 2000-2021 are multidisciplinary and take place in entirely new areas that Nobel could not have imagined developing (Charlton, 2007).

It can also be noticed that the average interval between announcing the discovery and awarding the Nobel Prize is growing, and thus the average age of the laureates. Because of that rule, many great scientists never won the Nobel Prize, even though they were nominated. Since the Nobel Prize cannot be awarded posthumously, it discourages many sciences and disavows the Nobel Prize (Fortunato, 2014).

Nevertheless, one important thing has changed: research disciplines that were not previously linked are now beginning to overlap and merge, and physicists, chemists, biologists, engineers, medics, computer scientists, and mathematicians are solving complex contemporary scientific problems together. One of these areas that are evolving at a rapid pace at the beginning of the 21st century is quantum biology - where quantum physicists, together with molecular biologists, are trying to explain numerous unknown and obscure phenomena in living cells. Over the past few decades, the Nobel Prize program has been slowly but steadily modified in a transparent and non-transparent manner. A transparent change is the establishment of the Nobel

Prize in Economics. The non-transparent change is that the Nobel Prize in Chemistry is transformed into the Nobel Prize in Biochemistry and Life Sciences. A detailed study includes evidence that the disciplines of chemistry and biochemistry cover different and generally scientifically unrelated fields today. An analysis of the Nobel Prizes in Chemistry in the period 2000-2021 encourages the Nobel Committee's program to monitor processes and changes in modern science in a transparent manner (Seeman & Restrepo, 2020).

So, if scientific research is increasingly interdisciplinary, isn't it time for the Nobel Prizes to follow their example and better reflect this trend? The Commission could introduce new categories and change them each year. Maybe one year, it will award the Nobel Prize for astrobiology, materials science, and geophysics, the following year for nanochemistry, artificial intelligence, and quantum biology. The boundaries between the sciences are being erased. Why not just reward the best research? This is not a new idea; physicists and biologists have collaborated fruitfully in the past. Long ago, in 1962, Crick (physicist) and Watson (biologist) were awarded for their discovery of the molecular structure of DNA, which helped solve one of the most important of all biological riddles (https://www.theguardian.com/commentisfree/2012/oct/08/nobel-prizes-need-shakeup)?

The desire to anticipate discoveries permeates almost all aspects of modern science, from individual scientists to publishers, from funding agencies to employment committees. A new and interdisciplinary field of “science of science” is emerging studies of the predictability of scientific discovery and its potential positive and negative impact on the scientific community (Clauset et al., 2017).

Conclusion

An analysis of the Nobel Prizes in Chemistry in the period 2000-2021 indicates that the largest number of prizes in this field are awarded for discoveries in biochemistry. The Nobel Prize in Chemistry is increasingly becoming the Nobel Prize in Biochemistry and Life Sciences. Suppose we extend this analysis to other scientific branches. In that case, similar results will be obtained because scientific branches are increasingly intertwined, and scientific disciplines that could not be assumed to appear and develop to that extent are formed. The fact is that modern research is very complex and has a multidisciplinary character. More and more researchers dealing with various scientific branches and disciplines participate in them. All this indicates the need to transform the Nobel Prize in Chemistry. It is necessary to erase the strict boundaries between the scientific branches for which the Nobel Prizes have been awarded so far, increase the number of scientists awarded the Nobel Prize, follow modern scientific research, and reward the most significant scientific achievements each year. This would contribute to the tremendous enthusiasm of scientists and would give a great incentive for even faster development of science.

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Conflict-of-Interest Statement

None.

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Chemia Naissensis, Vol 4, Issue 2, REVIEW ARTICLE, 11

Dobitnici Nobelove nagrade za hemiju u periodu 2000-2021.

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SAŽETAK

Nobelova nagrada za hemiju ima dugu istoriju od 1901. U prethodnom periodu došlo je do velikih promena i otkrića u oblasti hemije. Ovo je posebno intenzivirano u periodu 2000-2021. Došlo je do razvoja novih naučnih disciplina, od kojih su neke postale potpuno samostalne. Takođe, naučna otkrića su sve više multidisciplinarna i rezultat su rada velikih naučnih timova. Međutim, sve to ne prati povećanje broja oblasti za koje se dodeljuje Nobelova nagrada. Svrstavanje naučnih dostignuća u jednu od 6 oblasti za koje se dodeljuje Nobelova nagrada sve je teže, zbog njihove složenosti i multidisciplinarnosti. U periodu 2000-2021, najveći broj Nobelovih nagrada dodeljen je za istraživanja u oblasti biohemije (11), organske sinteze (4), razvoja novih metoda i materijala (4) i ostalo (3). Sve ovo ukazuje na dominaciju istraživanja u oblasti biohemije i nauke o životu i njihovu nezavisnost, što nije adekvatno praćeno promenama u strukturi i oblastima za koje se dodeljuju Nobelove nagrade. Nobelov komitet bi u bliskoj budućnosti trebalo da razmotri ovo pitanje i uskladi ga sa savremenim trendovima u nauci.

Ključne reči: Nobelove nagrade za hemiju, analiza

Chemia Naissensis, Vol 4, Issue 2, REVIEW ARTICLE, 12

Lauréats du prix Nobel de chimie pour la période 2000-2021

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RÉSUMÉ

Le prix Nobel de chimie a une longue histoire depuis 1901. Au cours de la période précédente, il y a eu des changements et des découvertes importants en chimie, particulièrement intensifiés au cours de la période 2000-2021. Il y a eu le développement de nouvelles disciplines scientifiques en chimie, dont certaines sont devenues complètement indépendantes. En outre, les découvertes scientifiques sont de plus en plus pluridisciplinaires et le fruit du travail de grandes équipes scientifiques. Cependant, tout cela ne s’accompagne pas d’une augmentation du nombre de domaines pour lesquels le prix Nobel est décerné. La classification des réalisations scientifiques dans l’un des six domaines pour lesquels le prix Nobel est décerné est de plus en plus délicate en raison de leur complexité et de leur multidisciplinarité. Au cours de la période 2000-2021, le plus grand nombre de prix Nobel ont été décernés pour la recherche dans le domaine de la biochimie (11), de la synthèse organique (4), du développement de nouvelles méthodes et matériaux (4) et dans d’autres domaines (3). Tout cela indique la prédominance de la recherche en biochimie et en sciences de la vie et son indépendance, qui ne s’accompagne pas suffisamment de changements dans la structure et les domaines pour lesquels les prix Nobel sont décernés. Dans un avenir proche, le Comité Nobel devrait examiner cette question et l’harmoniser avec les tendances scientifiques modernes.

Mots-clés : prix Nobel de chimie, analyse

Chemia Naissensis, Vol 4, Issue 2, REVIEW ARTICLE, 13

Лауреаты Нобелевской премии по химии за период 2000-2021 г.

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АННОТАЦИЯ

Нобелевская премия по химии имеет долгую историю с 1901 года. В предшествующий период произошли значительные изменения и открытия в химии, особенно активизировавшиеся в период 2000-2021 гг. Произошло развитие новых научных дисциплин в химии, некоторые из которых стали полностью самостоятельными. Также научные открытия все чаще становятся междисциплинарными и являются результатом работы больших научных коллективов. Однако все это не сопровождается увеличением количества направлений, за которые присуждается Нобелевская премия. Отнесение научных достижений к одной из шести областей, за которые присуждается Нобелевская премия, становится все более сложной из-за их сложности и междисциплинарности. В период 2000-2021 гг. Наибольший номер Нобелевских премий был присужден за исследования в области биохимии (11), органического синтеза (4), разработки новых методов и материалов (4) и др. (3). Все это свидетельствует о доминировании исследований в области биохимии и наук о жизни и их самостоятельности, что не сопровождается должным образом изменениями структуры и направлений, за которые присуждаются Нобелевские премии. В ближайшее время Нобелевский комитет должен рассмотреть этот вопрос и привести его в соответствие с современными тенденциями в науке.

Ключевые слова: Нобелевские премии по химии, анализ

Chemia Naissensis, Vol 4, Issue 2, REVIEW ARTICLE, 14

Nobelpreisträger für Chemie im Zeitraum 2000-2021

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ABSTRACT

Der Nobelpreis für Chemie hat eine lange Geschichte seit 1901. In der vorangegangenen Periode gab es bedeutende Veränderungen und Entdeckungen in der Chemie, besonders intensiviert im Zeitraum 2000-2021. In der Chemie entwickelten sich neue wissenschaftliche Disziplinen, von denen einige völlig unabhängig wurden. Außerdem sind wissenschaftliche Entdeckungen zunehmend multidisziplinär und das Ergebnis der Arbeit großer wissenschaftlicher Teams. All dies geht jedoch nicht mit einer Zunahme der Bereiche einher, für die der Nobelpreis verliehen wird. Die Einordnung wissenschaftlicher Leistungen in einen der sechs Bereiche, für die der Nobelpreis verliehen wird, wird aufgrund ihrer Komplexität und Multidisziplinarität immer schwieriger. Im Zeitraum 2000-2021 wurden die meisten Nobelpreise für Forschungen in den Bereichen Biochemie (11), organische Synthese (4), Entwicklung neuer Methoden und Materialien (4) und in anderen Bereichen (3) vergeben. All dies weist auf die Dominanz der Forschung in Biochemie und Biowissenschaften und ihre Eigenständigkeit hin, die nicht adäquat mit den Veränderungen in der Struktur und den Bereichen einhergeht, für die die Nobelpreise vergeben werden. In naher Zukunft sollte sich das Nobelkomitee mit diesem Thema befassen und es mit den modernen Trends in der Wissenschaft in Einklang bringen.

Schlüsselwörter: Nobelpreis für Chemie, Analyse

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Gas-chromatographic analysis of volatile compounds in different types of commercial alcoholic beverages

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ABSTRACT

In this study, volatile compounds were analysed in seven samples of different types of commercial alcoholic beverages available in stores in the Republic of Serbia. A total of 75 volatile compounds were detected by the gas chromatographic-mass spectrometric (GC/MS) method. Regarding the class of identified compounds, esters were the most dominant class identified in samples *Prirodna prepečenica*, *Metaxa* and *Whiskey*; *Tequila* and *Quince brandy* were dominated by higher alcohols, while in the samples *Pelinkovac* and *Travarica*, other classes of compounds were identified as the main. The major volatile compounds identified in tested samples were different. *Prirodna prepečenica* and *Travarica* were dominated by ethyl lactate; *Metaxa* was dominated by diethyl malate; *Quince brandy* had the highest amount of *n*-hexanol; most dominant compound in *Tequila* was benzyl alcohol; *Whiskey* was dominated by phenyl ethyl alcohol, while in *Pelinkovac trans*-thujone was identified as major compound. The results obtained in this study have shown that tested alcoholic beverages have different qualitative and quantitative compositions regarding the volatile compounds.

Keywords: alcoholic beverages, chemical composition, volatiles, GC-MS

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Introduction

Based on raw materials and production methods, alcoholic beverages can be divided into different classes: beer, wine, cider and distilled spirits. Spirits are made from many raw materials that contain sugar or starch, which is converted into sugar by malting. Various brandies are made from fruit mash (plums, cherries, apples, pears, apricots, and quinces); vodkas are produced from potatoes, tequila is distilled from a mash made from cactus, while the base of all types of whisky is malted grains. Quality and composition of spirits depends on the raw material that is used (Biernacka and Wardencki, 2012; Coldea et al., 2011; Hernandez-Gomez et al., 2005) and the production process (Arrieta-Garay et al., 2013; Lukić et al., 2011; Madrera et al., 2003; Madrera and Alonso, 2011; Matias-Guiu et al., 2016; Matijašević et al., 2019; Radeka et al., 2008; Soufleros et al., 2005; Spaho, 2017; Stamenković and Stojanović, 2020; Tsakiris et al., 2014). An important role in the organoleptic characteristics and quality of alcoholic beverages has various volatile compounds (such as alcohols, esters, volatile acids, terpenes, *etc*.) present in different concentrations. Besides volatile compounds, there are several different groups of non-volatile compounds that also contribute to the flavour of the spirits. Terpenes, which can be present in alcoholic beverages, are mainly derived from the raw material that is used for distillation, or they can be subsequently added to the beverage after the distillation to improve the flavour. Aside from ethanol (the most abundant compound besides water) and methanol (alcohol with toxic effect), higher alcohols that are formed during fermentation can also be detected in alcoholic beverages. The presence of higher alcohols can have both positive and negative impacts on the aroma, and flavour depending on concentration. Esters are aromatic compounds with a pleasant aroma, and therefore, the presence of these compounds has a positive effect on the aroma of spirits. Different groups of aroma compounds that can be detected in the brandies can originate from the fruit (primary aromatic compounds) or can be formed during alcoholic fermentation (secondary aromatic components), during the distillation process (tertiary aromatic compounds) and the maturation process (quaternary aromatic compounds) (Tešević et al., 2005).

As a part of our ongoing investigation on the composition of the volatile components of alcoholic beverages (Stamenković and Stojanović, 2020), this study aimed to determine the volatiles composition of seven different commercial brandies by applying the gas chromatography coupled with mass spectrometry (GC-MS).

Experimental

Seven samples were analyzed: *Prirodna prepečenica* (Takovo, Gornji Milanovac); *Tequila* Camina real gold (Tequila Cascahulin, Mexico); *Metaxa* (S.&E.&A. Metaxa, A.B.E., produce of Greece); *Quince brandy* (Simex Original); *Pelinkovac* (Gorki list); *Viski* (Longmorn) and *Travarica* (Podrumi manastira Tvrdoš).

Preparation of *rakija* for GC-MS analysis

Eighty millilitres of spirits were mixed with 80 mL of distilled water and 40 mL of CH_2Cl_2. Eight grams of NaCl was added, and the mixture was stirred on a magnetic stirrer for 30 minutes. The layers were separated into a separating funnel, and the organic layer was dried above anhydrous $MgSO_4$. The extract was concentrated to 1 mL on a vacuum evaporator and directly analyzed by gas chromatography-mass spectrometry (GC-MS) (Tešević et al., 2005).

GC-MS analysis

GC-MS analyses were performed on an Agilent 7890 gas chromatograph with a 7000B GC-MS-MS triple quadrupole system, operating in MS1 scan mode, and equipped with a fused-silica capillary column Agilent HP-5 MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The chromatographic analyses were carried out in the following conditions: He as carrier gas at a flow rate of 1.0 mL/min, GC oven temperature was kept at 50 °C for 2.25 min and programmed to 290 °C at a rate of 4 °C/min. One μL of the concentrated extract was injected at a split ratio of 40:1. The injector and interface operated at 250 and 300°C, respectively. Post run: back flash for 1.89 min, at 280 °C, with helium pressure of 50 psi. Ionization mode was an electronic impact at 70 eV. Mass range was set from 40 to 440 Da.

The percentage amounts of the separated compounds were calculated from the total ion chromatogram.

Identification of volatile compounds

Components were identified by comparison of their mass spectra with those of Wiley 6, Adams (2007), NIST 11 and Essential oils libraries, applied on Agilent Mass Hunter Workstation (B.06.00) and AMDIS (2.1, DTRA/NIST, 2011) software and confirmed by comparing of calculated retention indexes (relative to C_8-C_{40} *n*-alkanes) with the literary values of the retention indices.

Results and Discussion

In the samples subjected to this study, a total of 75 compounds were identified and presented in Table 1. In the individual samples of *Prirodna prepečenica* (L1), *Tequila* (L2), *Metaxa* (L3), *Quince brandy* (L4), *Pelinkovac* (L5), *Whiskey* (L6) and *Travarica* (L7), a total of 30, 17, 16, 20, 18, 20 and 33 volatile compounds were identified, respectively. Esters were the most dominant class of the compounds identified in samples L1, L3 and L6; alcohols were dominant in L2 and L4, while in the samples L5 and L7, components that do not belong to alcohols or esters were the most abundant compounds. It can be noticed (Table 1), that furfural was the only compound that was present in all samples with the different contributions. A high concentration of furfural may have a toxic effect on the human organisms, while in low concentration, the presence of this compound contributes to the aroma and flavour of fruit distillates. The most dominant compound in sample L1 was ethyl lactate (22.8%), followed by benzyl alcohol (22.4%). Ethyl lactate was also identified as a major compound in sample L7 with the contribution of 15.2%. With 33 identified components, *Travarica* (L7) seems to possess the wealthiest volatile composition, with 10 compounds that were exclusive to this sample. The sample L5 differs from others because in this sample, higher alcohols were not detected at all. Tributyl acetylcitrate was the only ester that was identified in this sample, with the contribution of 15.3%. The most dominant in this sample were compounds that do not belong either to alcohols or esters (65.1%), with *trans*-thujone (19.8%) as a major component. The main difference between *Pelinkovac* and all the other samples is that in this sample, terpenes were qualitatively and quantitatively the largest group of compounds. Higher alcohols were quantitatively the largest group of the volatile aroma compounds identified in the sample L4 (42%). The most dominant compound in this sample was *n*-hexanol with the contribution of 40.5%, while in the samples L1 and L7, hexanol was present in the concentration of 2.8% and 0.5%, respectively and not even detected in the samples L2, L3, L5 and L6. Sample L6 (*Whiskey*) was dominated by phenyl ethyl alcohol with the contribution of 27.9%. This component is responsible for the rose-like aroma in spirits (Ferrari et al., 2004). Sample L3 (*Metaxa*) could be distinguished from the other samples by the highest relative amount of esters (75.9%) with diethyl malate as the main component (44.9%). This ester was detected only in the *Metaxa* sample, and its presence contributes to the sweet, caramellike odour of the spirit. Regarding the *Tequila* sample (L2), the most dominant compounds were alcohols with benzyl alcohol as the main component (14.7%). This compound contributes to the pleasant sweet, floral aroma. By comparing the chemical composition of *Prirodna prepečenica* with our previously published results of homemade plum brandy (Stamenković and Stojanović, 2020), it can be noticed that regarding the major compound, all tree samples were dominated by ethyl lactate and with similar contribution (24.3% in the sample plum “ranka”, 20.1% in distillate obtained from plum “čačanska rodna” and 22.8% in *Prirodna prepečenica*). On the other hand, Agalarov et al. (2017) were investigating quince brandy as one of the traditional fruit brandies produced in Azerbaijan, and the obtained results were quite different compared to ours. In the sample from Azerbaijan, the most dominant volatile compounds were ethyl acetate and acetaldehyde, two compounds that were not even detected in our sample.

Table 1. Chemical composition of commercial alcoholic beverages

				Content (%)						
No	RI	RN	Compound	L1	L2	L3	L4	L5	L6	L7
1	762	760	Isopentyl alcohol	-	-	-	0.9	-	-	-
2	765	762	Pentanol	0.6	-	-	-	-	-	-
3	766	766	Cyclopentanone	-	3.1	-	-	-	-	-
4	775	778	Ethyl butanoate	0.7	1.9	1.0	2.6	-	0.3	-
5	780	780	Dihydro-2-methyl-3-furanone	-	2.2	-	-	-	-	-
6	794	798	Ethyl lactate	22.8	12.7	9.9	4.0	-	-	15.2
7	810	815	Furfural	0.6	1.8	1.7	4.8	0.6	0.9	0.6
8	852	858	*n*-Hexanol	2.8	-	-	40.5	-	-	0.5
9	856	872	1-Butoxy-1-ethoxyethane	-	-	-	-	-	-	0.6
10	861	867	Isopentyl acetate	0.3	0.5	-	2.3	-	0.9	0.4
11	926	924	α-Pinene	-	-	-	-	-	-	4.8
12	940	946	Camphene	-	-	-	-	-	-	1.0
13	948	955	1,1-Diethoxy-3-methyl-butane	-	-	0.5	-	-	-	-
14	954	959	Benzaldehyde	1.7	-	0.5	0.7	-	-	-
15	958	957	2-Acetylfuran	-	2.6	-	-	-	-	-

16	968	977	1-(1-ethoxyethoxy)-pentane	-	-	-	-	-	-	1.3
17	995	997	Ethyl hexanoate	0.6	0.7	0.9	1.0	-	1.0	0.4
18	1009	1007	Hexyl acetate	-	-	-	2.3	-	-	-
19	1018	1020	*p*-Cymene	-	-	-	-	0.4	-	1.6
20	1024	1024	Limonene	-	-	-	-	-	-	1.1
21	1025	1026	Eucalyptol	-	-	-	-	1.0	-	7.7
22	1030	1034	Benzyl alcohol	4.3	14.7	1.2	-	0.8	-	-
23	1069	1067	*cis*-Linalool oxide	0.8	0.9	-	-	-	-	0.2
24	1085	1084	*trans*-Linalool oxide	1.2	0.6	-	-	-	-	-
25	1096	1095	Linalool	-	1.1	-	-	0.8	-	0.6
26	1100	1100	*cis*-Thujone	-	-	-	-	8.1	-	7.1
27	1110	1115	Phenyl ethyl alcohol	1.3	2.6	0.4	-	6.7	27.9	-
28	1010	1012	*trans*-Thujone	-	-	-	-	19.8	-	2.3
29	1038	1041	Camphor	-	-	-	-	-	-	2.7
30	1048	1048	Menthone	-	-	-	-	3.3	-	-
31	1058	1058	*iso*-Menthone	-	-	-	-	1.8	-	-
32	1062	1065	Borneol	-	-	-	-	-	-	1.7
33	1063	1061	*neo*-Menthol	-	-	-	-	0.7	-	-
34	1164	1163	4-Ethyl-phenol	0.4	-	-	-	-	-	-
35	1167	1169	Ethyl benzoate	4.8	-	-	-	-	-	-
36	1070	1067	Menthol	-	-	-	-	3.2	-	-
37	1075	1074	Terpinen-4-ol	-	-	-	-	5.3	-	0.8
38	1173	1170	Octanoic acid	-	-	-	-	-	2.0	-
39	1177	1181	Diethyl succinate	4.4	-	10.0	0.8	-	1.5	12.7
40	1188	1186	α-Terpineol	1.2	3.9	1.4	-	0.8	-	1.1
41	1193	1194	Ethyl octanoate	1.5	4.1	4.8	5.7	-	8.6	0.7
42	1204	1204	Verbenone	-	-	-	-	-	-	6.0

43	1225	1246	Benzaldehyde diethylacetal	0.3	-	-	-	-	-	-
44	1226	1233	5-Hydroxymethylfurfural	-	-	14.0	-	6.0	-	-
45	1264	1271	Diethyl malate	-	-	44.7	-	-	-	-
46	1268	1266	Ethyl salicylate	0.8	-	-	-	-	-	-
47	1287	1289	*trans*-Sabinyl acetate	-	-	-	-	9.7	-	-
48	1287	1289	Thymol	-	-	-	-	-	-	5.9
49	1292	1295	Ethyl nonanoate	0.3	-	-	-	-	-	-
50	1355	1356	Eugenol	2.9	-	-	-	-	-	-
51	1370	1364	Decanoic acid	1.3	-	-	-	-	3.4	-
52	1382	1383	(*E*)-β-Damascenone	-	-	-	1.0	-	-	-
53	1392	1392	Ethyl decanoate	3.6	8.7	3.5	10.5	-	20.9	0.3
54	1396	1393	Vanillin	0.4	-	0.5	-	-	0.5	-
55	1425	1422	(*E*)-α-Ionone	-	-	1.2	-	-	-	-
56	1463	1465	(*E*)-Ethyl cinnamate	1.8	-	-	-	-	-	-
57	1484	1487	(*E*)-β-Ionone	-	-	-	1.4	-	-	-
58	1561	1565	Dodecanoic acid	-	-	-	-	-	0.7	-
59	1570	1569	γ-Undecalactone	-	-	-	2.2	-	-	-
60	1590	1593	Ethyl dodecanoate	2.8	3.4	1.1	4.8	-	17.0	-
61	1640	1641	Isoamyl decanoate	-	-	-	-	-	0.3	-
62	1657	1655	Syringaldehyde	-	-	-	-	-	1.1	-
63	1671	1671	*n*-Tetradecanol	-	-	-	-	-	1.8	-
64	1789	1795	Ethyl tetradecanoate	0.6	-	-	0.6	-	3.6	-
65	1873	1874	*n*-Hexadecanol	-	-	-	-	-	2.9	-
66	1967	1977	Ethyl 9-hexadecenoate	-	-	-	-	-	5.7	-
67	1988	1993	Ethyl hexadecanoate	3.2	1.0	-	1.3	-	8.0	0.3
68	2050	2056	Manool	-	-	-	-	-	-	1.6
69	2156	2163	Ethyl linoleate	0.7	-	-	0.5	-	-	-

70	2162	2169	Ethyl oleate	1.1	-	-	-	-	0.4	-
71	2162	2173	Ethyl linolenate	-	-	-	-	-	-	1.1
72	2255	2253	Tributyl acetylcitrate	-	-	-	-	15.3	-	-
73	2486	2500	Pentacosane	-	-	-	-	1.1	-	0.5
74	2586	2600	Hexacosane	-	-	-	-	1.4	-	0.6
75	2685	2700	Heptacosane	-	-	-	-	1.1	-	-
Number of constituents				30	17	16	20	18	20	31
Total identified				95.0	93.2	96.2	88.5	80.4	96.9	83.0
Alcohols				34.2	44.0	0.5	42.0	/	20.1	2.1
Esters				50.0	33.0	75.9	36.4	15.3	68.2	31.1
Others				10.8	16.2	19.8	10.1	65.1	8.6	49.8

Compounds listed in order of elution on an HP-5 MS column. RI: experimentally determined retention indices on the mentioned column of a homologous series of *n*-alkanes C_8-C_{40}; RN: NIST Chemistry WebBook Retention indices; -: not detected. Samples: L1-*Prirodna prepečenica*, Takovo; L2-*Tequila*, Camina real gold; L3-*Metaxa*; L4- *Quince brandy*, Simex Original; L5-*Pelinkovac*, Gorki list; L6-*Whiskey*, Longmorn; L7-*Travarica*, Podrumi manastira Tvrdoš.

Conclusion

Detailed GC-MS analyses of extracts of the seven tested brandies were performed, and the qualitative and quantitative composition of the tested brandies were compared. Tested brandies were dominated by a different class of compounds. The major volatile compounds identified in tested samples were different, which was expected bacause each of the samples had its unique and characteristic aroma.

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Conflict-of-Interest Statement

All authors declare that they have no conflict of interest.

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Gasno-hromatografska analiza isparljivih jedinjenja u različitim vrstama komercijalnih alkoholnih pića

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SAŽETAK

U ovom radu analiziran je hemijski sastav lako isparljivih jedinjenja sedam različitih vrsta komercijalnih alkoholnih pića, koja se mogu nabaviti u prodavnicama u Republici Srbiji. Primenom gasne hromatografije sa masenom spektrometrijom (GC/MS) identifikovano je ukupno 75 jedinjenja. Identifikovane komponente pripadaju različitim klasama jedinjenja, pri čemu su estri bili najdominantnija klasa u uzorcima Prirodna prepečenica, Metaxa, Whisky, Tequila i rakija Dunja imale su najveći procenat viših alkohola, dok su u uzorcima Pelinkovac i Travarica najzastupljenija jedinjenja koja ne pripadaju ni estrima ni alkoholima. Što se tiče glavnih komponenti u ispitivanim uzorcima pića, u uzorcima Prirodna prepečenica i Travarica kao glavna komponenta je identifikovan etil-laktat; Metaksa je uzorak u kojem je najzastupljeniji dietil-malat; rakija Dunja je imala najviše *n*-heksanola; benzil-alkohol je bio glavna komponenta u uzorku Tequila; Whisky je piće u kojem je kao kao dominantno isparljivo jedinjenje identifikovan fenil-etil-alkohol, dok je *trans*-tujon bio najzastupljenija komponenta u uzorku Pelinkovc. Dobijeni rezultati pokazali su da analizirani uzorci alkoholnih pića imaju različit kvalitativni i kvantitativni sastav.

Ključne reči: alkoholna pića, hemijski sastav, isparljive materije, GC-MS

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Analyse par chromatographie en phase gazeuse des composés volatils dans les différents types de boissons alcoolisées commerciales

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RÉSUMÉ

Dans ce travail, a été analysée la composition chimique des composés volatils dans les sept échantillons de différentes catégories de boissons alcoolisées commerciales qui sont disponibles dans les magasins en République de Serbie. Au total, les soixante-quinze composés volatils ont été détectés à l'aide de la méthode de chromatographie en phase gazeuse-spectrométrie de masse (GC/MS). En ce qui concerne la classe de composés identifiés, les esters étaient la classe la plus dominante dans les échantillons de *Prirodna prepečenica*, de *Metaxa* et du *Whiskey*; *La tequila* et *Le coing brandy* étaient dominés par des alcools supérieurs, tandis que dans les échantillons de *Pelinkovac* et de *Travarica*, d'autres classes de composés ont été identifiées comme principales. Les principaux composés volatils identifiés dans les échantillons testés de boissons étaient différents: les échantillons de *Prirodna prepečenica* et de *Travarica* étaient dominés par le lactate d'éthyle; celui de *Metaxa* était dominé par le malate de diéthyle; *Le coing brandy* avait la plus grande quantité de *n*-hexanol; le composé le plus dominant dans *La tequila* était l'alcool benzylique; *Le whisky* était dominé par l'alcool phényléthylique, tandis que dans l'échantillon de *Pelinkovac*, la *trans*-thuyone était identifiée comme composé principal. Les résultats obtenus dans cet examen ont montré que les boissons alcoolisées testées disposent des compositions qualitative et quantitative différentes concernant les composés volatils.

Mots-clés : boissons alcoolisées, composition chimique, volatiles, GC-MS

Chemia Naissensis, Vol 4, Issue 2, RESEARCH ARTICLE, 27

Газохроматографический анализ летучих соединений в различных типах коммерческих алкогольных напитков

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АННОТАЦИЯ

В этом исследовании летучие соединения были проанализированы в семи образцах различных видов коммерческих алкогольных напитков, доступных в магазинах Республики Сербия. Всего 75 летучих соединений было обнаружено методом газовой хроматографии-масс-спектрометрии (ГХ/МС). Что касается класса идентифицированных соединений, то преобладающим классом, идентифицированным в образцах “Prirodna prepečenica”, “Metaxa” и “Whisky”, были сложные эфиры; В текиле и бренди из айвы преобладали высшие спирты, в то время как в образцах “Pelinkovac” и “Travarica” в качестве основного были определены другие классы соединений. Основные летучие соединения, идентифицированные в испытанных образцах, были разными. В пробах „Prirodna prepečenica“ и „Travarica“ преобладал этиллактат; В пробе „Metaxa“ преобладает диэтилмалат; В бренди из айвы было больше всего н-гексанола; наиболее доминирующим соединением в текиле был бензиловый спирт; В виски преобладал фенилэтиловый спирт, в то время как в пелинковце транс-туйон был определен как основное соединение. Результаты, полученные в этом исследовании, показали, что испытанные алкогольные напитки имеют различный качественный и количественный состав в отношении летучих соединений.

Ключевые слова: алкогольные напитки, химический состав, летучие вещества, ГХ-МС

Chemia Naissensis, Vol 4, Issue 2, RESEARCH ARTICLE, 28

Gaschromatographische Analyse flüchtiger Verbindungen in verschiedenen Arten handelsüblicher alkoholischer Getränke

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ABSTRACT

In dieser Studie wurden flüchtige Verbindungen in sieben Proben verschiedener Arten von handelsüblichen alkoholischen Getränken analysiert, die in Geschäften in der Republik Serbien erhältlich sind. Insgesamt wurden 75 flüchtige Verbindungen mit Hilfe der Gaschromatographisch-Massenspektrometrie (GC/MS) nachgewiesen. Die identifizierten Komponenten gehören verschiedenen Verbindungsklassen an, so waren Ester die vorherrschende Klasse, die in den Proben Prirodna prepečenica, Metaxa und Whisky gefunden wurden; bei Tequila und Quittenschnaps dominierten höhere Alkohole, während in den Proben Pelinkovac und Travarica andere Verbindungsklassen, die weder zu den Estern noch zu den höheren Alkoholen gezählt werden können, dominieren. Die Hauptkomponenten, die in den untersuchten Proben identifiziert wurden, waren in Prirodna prepečenica und Travarica Ethyllactat, in Metaxa dominierte Diethylmalat, in Quittenschnaps n-Hexanol, in Tequila Benzylalkohol und in Whiskey Phenylethylalkohol, während in Pelinkovac Trans-Thujon als Hauptkomponente identifiziert wurde. Die Ergebnisse dieser Studie haben gezeigt, dass die untersuchten alkoholischen Getränke eine unterschiedliche qualitative und quantitative Zusammensetzung der flüchtigen Verbindungen aufweisen.

Schlüsselwörter: alkoholische Getränke, chemische Zusammensetzung, flüchtige Stoffe, GC-MS

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Analyses of structure and thermal properties of synthesized crosslinked poly(1-vinyl-2-pyrrolidone-*co*-vinyl acetate) hydrogels

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ABSTRACT

This paper describes the process for the synthesis of chemically crosslinked copolymer hydrogels based on monomer 1-vinyl-2-pyrrolidone and comonomer vinyl acetate, using the varied content of ethylene glycol dimethacrylate as a crosslinker, by the free radical polymerization method with thermal initiation. The content of unreacted reactants after poly(1-vinyl-2-pyrrolidone-co-vinyl-acetate), p(VP-VA), hydrogels synthesis was examined using high-pressure liquid chromatography (HPLC) method. Structure characterization of the obtained p(VP-VA) hydrogels was performed using the Fourier transform infrared spectroscopy (FTIR). In this study, the influences of crosslinker content and temperature on the swelling behaviour of p(VP-VA) were studied. Quantities of unreacted comonomers and crosslinker, calculated in relation to the initial amount present in the reaction mixture, confirmed their successful conversion into p(VP-VA) hydrogels. These unreacted values of 1-vinyl-2-pyrrolidone (in range of 0.605-1.609%), vinyl acetate (in range of 2.486-4.798%), and ethylene glycol dimethacrylate (in range of 0.889-3.240%) were within acceptable limits, and they were removed from the final products. FTIR spectra were verified that the copolymerization process was performed, and chemically crosslinking of polymer chains occurred by breaking double bonds from the reactants. Obtained crosslinked copolymers could be classified in the class of negative-thermosensitive hydrogels because they can swell and pass through a phase transition when heated from the swollen state at 25ºC to contracted state at 80ºC.

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Keywords: hydrogel, chemically crosslinking, 1-vinyl-2-pyrrolidone, vinyl acetate, swelling, thermosensitivity

Introduction

The monomer 1-vinyl-2-pyrrolidone, VP, has widely applied polymer for the production of homopolymer poly(1-vinyl-2-pyrrolidone) (p(VP)), heteropolymers and copolymers with acrylic acid, acrylates, vinyl acetate, and also with other monomers (Teodorescu and Bercea, 2015). It has unique, remarkable physical and chemical properties, such as chemical stability, good solubility in water and many organic solvents, affinity to complex both hydrophobic and hydrophilic substances, biocompatibility, nontoxicity. Thanks to these properties it is suitable as a biomaterial in various significant pharmaceutical and medical applications and many non-medical applications (*i.e.*, environmental, optical, electrical). Crosslinked polymers based on VP can be obtained by a series of processes as: a) slightly crosslinked (by γ-radiation) (Henglein, 1959) or by treatment with persulphate (Anderson et al., 1979) or with hydrazine and hydrogen peroxide (Schildknecht, 1953), b) more densely crosslinked (by copolymerization with crosslinkers) (Atkins et al., 1972); c) highly crosslinked products, obtained by popcorn or proliferous polymerization (Haaf et al., 1985).

Vinyl acetate, VA, is used as a monomer to synthesize poly(vinyl acetate), p(VA), and many copolymers. It is used as wood glue, known as "white glue", as paper glue, and yellow "carpentry glue", thanks to strong, flexible bonds and non-acidic nature. Also, it is used in paper coatings, paints, and other industrial coatings, as a binder in the nonwoven fabric in glass fibers, filter paper, and textile finishing.

Poly(1-vinyl-2-pyrrolidone-vinyl acetate), p(VP-VA), as a well-known copolymer, was usually produced as a white or yellowish-white powder, or colorless or yellowish transparent liquid (Ashland, 2022). The pharmaceutical industry has used it for decades, *i.e.*, as a processing aid in the production of granules and tablets, as a binder in direct compression, in film coatings on tablets, as a protective layer, and subcoat for tablet cores. Due to the fact that there is no irritation for skin and eyes, it has been mainly used for hair care products as a finalizing agent, film-forming agent, and viscosity regulator. Two commercial products of the p(VP-VA) copolymer are available in the market having the pharmaceutical quality required by the Pharmacopoeias (the European

Pharmacopeia (Ph. Eur. 10), the USP-NF monograph “Copovidone”, Japanese Pharmaceutical Excipients) (Bühler, 2005).

Water-soluble p(VP-VA) copolymer contains the two components, VP and VA, in different ratios (60:40, 50:50, 70:30, 30:70, 35:65) and a wide range of molecular weights (Ashland, 2022). According to EP 0104042 (Eugene and James, 1982), VP and VA comonomers were polymerized using free radical initiator *tert*-butylperoxypivalate in a solvent consisting of water, isopropyl alcohol, secondary butyl alcohol, or mixtures thereof. Also, p(VP-VA) copolymer was produced by free radical polymerization method in 2-propanol with an organic peroxide as an initiator (Bühler, 2005). This copolymer is slightly more hydrophobic compared to homopolymer p(VP) because its VA comonomer is not soluble in water.

The powder p(VP-VA) copolymer is a spray-dried product in the form of deformed or partly broken hollow spherical particles. This irregular structure is one of the important reasons for the excellent dry binding properties of powder p(VP-VA) copolymer in the direct compression technology of tablets (Bühler, 2005).

Based on scientific opinion on the safety, the p(VP-VA) copolymer is safe for application in FDA approved food supplements, as a food additive, *e.g.*, in tablet form as a binding/coating agent in an amount of up to 10% of weight per tablet weight of 1000 mg (D’Amelia et al., 2019). The p(VP-VA) copolymers have found great application in the cosmetic industry, primarily in personal care products, in the manufacture of hair care products (Personal Care Products Council, 2017; Rothe et al., 2011).

The resins p(VP-VA) were produced as linear, random copolymers by the free-radical polymerization of the varying monomers ratios from 70/30 to 30/70 vinyl acetate to vinylpyrrolidone as 50% w/w ethanolic solutions (Browne et al., 2020). The commonly used p(VP-VA) polymer in amorphous solid dispersion systems has a VP:VA ratio 6:4, but it would be interesting to examine the effect of different ratios (*i.e.*, 4:6 and 5:5) for improvement the commercially used polymers.

Risk Assessment is important for VP to all environmental compartments for production, processing, and use of VP and release of VP during use of polymers which contain residual VP monomer, according to European Union Report (European Commision, 2003).

The term “hydrogel” is used to describe two- or multicomponent systems consisting of a three-dimensional network of polymer chains and water or fluid that fills the space between macromolecules. Hydrogel networks can absorb different amounts of water or fluids within their structure, usually swell to equilibrium, but without dissolution (Gulrez et al., 2011). Hydrogels that show changes in swelling under the action of external stimuli from the environment, such as pH, temperature, light intensity, belong to the group of stimuli sensitive hydrogels, or "intelligent" materials (Ilić-Stojanović et al., 2017).

This work aims to synthesize chemically crosslinked copolymer hydrogels of 1-vinyl-2-pyrrolidone with 10 mol% of vinyl acetate by the free radical polymerization method with thermal initiation and their structure characterization, as well as the swelling behavior in relation to the temperature changes.

Experimental

Reagents

1-Vinyl-2-pyrrolidone, VP, ≥99.0% (Merck KGaA, Darmstadt, Germany), vinyl acetate, VA, ≥99.0% (Merck KGaA, Darmstadt, Germany), ethylene glycol dimethacrylate, EGDM, 97.0% (Fluka Chemical Corp, CH), 2,2'-azobis(2-methylpropionitrile), 98.0% (Acros Organics, New Jersey, USA), methanol, 99.5% (Merck KGaA), and methanol HPLC grade, 99.9% (Chromasolv, Sigma-Aldrich Chemie GmbH, DE). All chemicals were used as received.

Hydrogel synthesis

Polymerization of 1-vinyl-2-pyrrolidone monomers and vinyl acetate comonomers (10 mol%, relative to the amount of 1-vinyl-2-pyrrolidone monomer) was performed using the free radical polymerization method. Various amounts of ethylene glycol dimethacrylate (1.0; 1.5; 2.0; 2.5 and 3.0 mol% relative to the total amount of monomer) were used for crosslinking of long-chain polymer chains. The reactants were dissolved in methanol, and the 2,2'-azobis(2-methylpropionitrile) as initiator was added in the reaction mixtures (2.7 mol%). After homogenization and dissolution of the reactants, the reaction mixtures were injected into glass ampoules, which were closed after that. The polymerization process for all samples was thermally initiated, according to the following temperature regime: 30 min at 70°C, 120 min at 80°C, and 90 min at 85°C. After cooling, the obtained copolymer hydrogels poly(1-vinyl-2-pyrrolidone-*co*-vinyl acetate) were separated from glass ampoules in the form of long cylinders and cut into smaller cylinders.

Extraction of unreacted compounds

The hydrogels p(VP-VA) were then immersed in methanol (60 cm^3 of methanol per 1.0 g of hydrogel) and stirred for 48 h at room temperature to extract all unreacted compounds and any impurities. After treatment with methanol, the p(VP-VA) hydrogels were successively immersed in fresh methanol/distilled water solutions: 80/20%, 60/40%, 40/60%, 20/80%, and 0/100%, v/v, for 24 h, to gradually washed the methanol from obtained products. Purified hydrogels p(VP-VA) were dried in an oven at 40°C to constant masses, after which they were subjected to further characterization.

Characterization of synthesized hydrogels

Analysis of unreacted contents of comonomers and crosslinker

Analyses of the qualitative and quantitative composition of unreacted comonomers of VP and VA and EGDM crosslinker, from methanolic extracts decanted after synthesis of p(VP-VA) hydrogels, were performed by using high-pressure liquid chromatography (HPLC) method. For these analyses, the construction of calibration curves is necessary using standard substances. Firstly, three series of standard solutions substances (VP, VA, and EGDM) with known concentrations (0.005-1.000 $mg\cdot cm^{-3}$) were prepared in methanol as solvent. All samples for HPLC analysis were filtered through a cellulose membrane filter with a pore diameter of 0.45 μm and analyzed using defined conditions. The HPLC Agilent 1100 Series is equipped with a DAD 1200 detector with adjustable absorption wavelength and automatic sampling of the adjusted injected volume. The detector was set to wavelengths of 205 nm and 209 nm. The ZORBAX Eclipse XDB-C18 column, 4.6 × 250 mm, 5 μm, was applied. The mobile phase (eluent) was: (A) methanol HPLC purity, 80% and (B) redistilled water, 20%. The column was thermostated at 30°C, the flow rate of the mobile phase was 1 $cm^3\cdot min^{-1}$, and the analysis time was 10 min. The injected volumes of the analyzed samples were 10 μl each. The obtained data for standard solutions and methanolic extracts decanted after p(VP-VA) synthesis were processed using Agilent ChemStation software.

Fourier transform infrared spectrophotometry (FTIR)

The FTIR spectra of liquid VP and VA comonomers were measured using the capillary film method between two polished CaF_2 plates. FTIR spectra of a synthesized sample of p(VP-

VA) xerogels with 10 mol% of the VA comonomer and 3.0 mol% of EGDM were ground to powder state in an amalgamator (WIG-L-BVG, 31210-3A, USA) and then recorded by the thin transparent pellets technique with potassium bromide of spectroscopic purity, after vacuuming and pressing under a pressure of about 200 MPa. All samples were recorded on an FTIR spectrophotometer Bomem Hartmann & Braun MB-series (Baptiste, Canada) in the wavenumbers range from 4000 to 400 cm^{-1}. Win-Bomem Easy software was used to process the recorded FTIR spectra of the reactants and the resulting products.

Thermosensitivity analysis

The temperature sensitivity of the synthesized p(VP-VA) hydrogels was examined by monitoring the change of the equilibrium swelling degree with increasing temperature in the range from 25ºC to 80ºC in a distilled water, which pH value was 6.0, measured using pH meter (HI9318-HI9219, Hanna, Portugal). The swelling process of p(VP-VA) samples was monitored gravimetrically. Firstly, the samples of dried hydrogels (xerogels) p(VP-VA) with 10 mol% VA and 1.0; 1.5; 2.0; 2.5 and 3.0 mol% of EGDM as a crosslinker, were immersed into the fluid at a temperature of 25ºC. The masses of the swollen samples were measured at a specific time until equilibrium was reached at a defined temperature. Changes in the swelling degree, α, and the equilibrium swelling degree, α_e, were calculated using equations (1) and (2), respectively, as the ratio of the mass of absorbed fluid and the xerogels mass:

$$\alpha = \frac{m_t - m_0}{m_0} \quad (1)$$

$$\alpha_e = \frac{m_e - m_0}{m_0} \quad (1)$$

m_o is the xerogel mass, m_t is the mass of the swollen hydrogel at the time t, and m_e is the mass of the hydrogel swollen at equilibrium state.

Results and Discussion

Synthesis of copolymer hydrogels p(VP-VA)

Free radical polymerization, as the most probable mechanism, was applied for the synthesis of a series of poly(1-vinyl-2-pyrrolidone-*co*-vinyl acetate) copolymer hydrogels with 10 mol%

vinyl acetate as comonomer and 1.0; 1.5; 2.0; 2.5 and 3.0 mol% EGDM as crosslinker. The reaction of free radical polymerization was initiated by the homolytic decomposition of the initiator 2,2`-azobis(2-methylpropionitrile) at a temperature higher than 70ºC. Produced primary radicals were transferred by adding the primary active center of the initiator most probable to the unsaturated vinyl groups of the 1-vinyl-2-pyrrolidone and the vinyl acetate molecules. Linear chains were probably crosslinked from two vinyl groups from the ethylene glycol dimethacrylate molecule during the propagation phase. The crosslinking between the linear chains was possibly performed thanks to two vinyl groups in the EGDM molecule. After the termination phase of the polymerization process, synthesized p(VP-VA) copolymer hydrogels were formed, with a possible structure presented at Figure 1(a). All copolymer hydrogels p(VP-VA) samples were of stable shape and soft consistency. The appearance of samples of synthesized copolymer hydrogels p(VP-VA) with 1.0 and 2.5 mol% of EGDM crosslinker was presented in Figures 1(b) and 1(c). Synthesized p(VP-VA) hydrogels were characterized in terms of chemical structure by the FTIR method, the amount of unreacted reactants during synthesis was calculated, and the swelling degree was examined depending on the surrounding temperature.

Analysis of unreacted contents of comonomers and crosslinker

The series of standards solutions with known amounts of VP, VA, and EGDM were prepared for the calibration curve construction and analyzed by the HPLC method. The quantification of the amount of the released VP and EGDM as a function of time was performed using the previously published method (Ilić-Stojanović and Eraković, 2019).

Calibration curve for 1-vinyl-2-pyrrolidone

The HPLC chromatogram of the VP standard solution had a retention time of R_t=3.191 min, and the maximum wavelength of UV absorption at λ_{max}=209 nm. The constructed calibration curve for VP was linear for peak areas in the range of 0.007-0.804 mg·cm^{-3}, *i.e.*, for peak areas in the range of 126-12200 mAU·s (Figure 2(a)); for this linearity equation (2) applies (Ilić-Stojanović and Eraković, 2019):

$$A_{209nm} = 124{,}20 + 16708{,}57 \cdot c_{VP} \qquad (2)$$

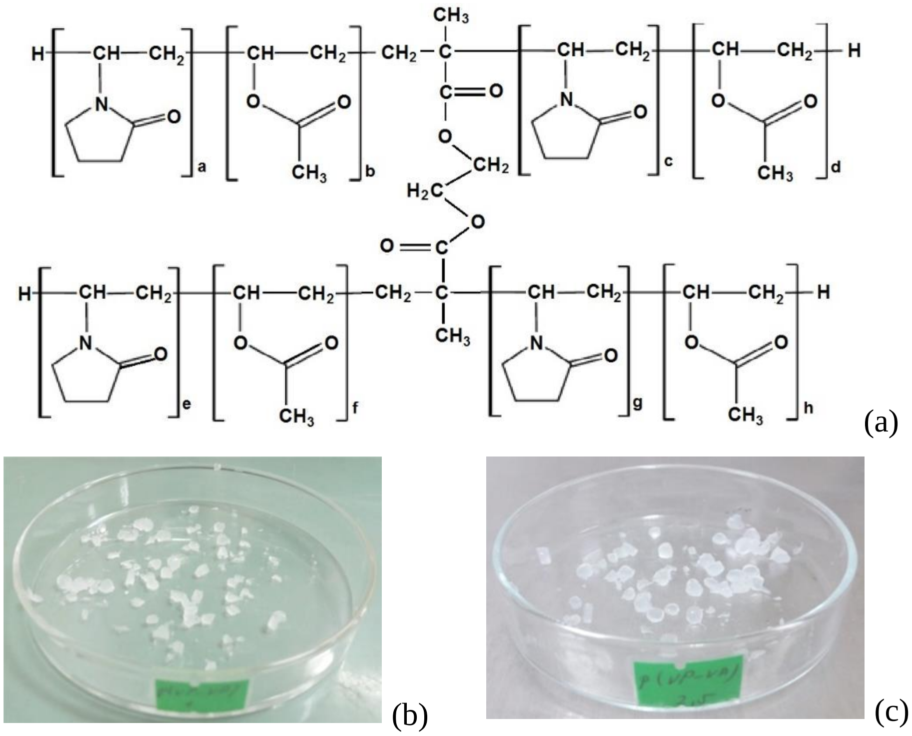


Figure 1. (a) Potential structural formula of crosslinked poly(1-vinyl-2-pyrrolidone-co-vinyl acetate) hydrogel. The appearance of synthesized hydrogels with 10 mol% vinyl acetate comonomer with (b) 1.0 mol% of EGDM as a crosslinker, and (c) 2.5 mol% of EGDM.

A_{209nm} is the peak area at λ_{max}=209 nm in mAU·s, c_{VP} is the concentration of VP in mg·cm^{-3}, the linear correlation coefficient is R^2=0.999, and the standard deviation of the blank is σ=106,408. The limit of detection, LoD, as the smallest amount or concentration of the analyte in the test sample that can be reliably distinguished from zero, was 0.0191 mg·cm^{-3}. The limit of quantitation (LoQ), as the lowest concentration of the analyte that can be determined with an acceptable repeatability and trueness, was 0.0637 mg·cm^{-3}.

Calibration curve for ethylene glycol dimethacrylate

The HPLC chromatogram of the EGDM standard solution under given conditions has a retention time R_t=6.125 min, and the UV spectrum shows the maximum absorption at λ_{max}=205 nm. The dependence of the peak area on the EGDM concentration is linear in the range of 0.005-0.3 mg·cm^{-3}, *i.e.*, for peak areas in the range of 300-18000 mAU·s. Equation (3) applies to the linear part of the constructed calibration curve for EGDM (Figure 2(b)) (Ilić-Stojanović and Eraković, 2019):

$$A_{205nm} = -19{,}34 + 76188{,}09 \cdot c_{EGDM} \tag{3}$$

where A_{205nm} is the peak area (mAU·s) at λ_{max}=205 nm, c_{EGDM} is the content of EGDM (mg·cm^{-3}), the linear correlation coefficient is R^2=0.999, σ=25.176, LoD=9.91·10^{-4}mg·cm^{-3}, and LoQ=0.0033 mg·cm^{-3}.

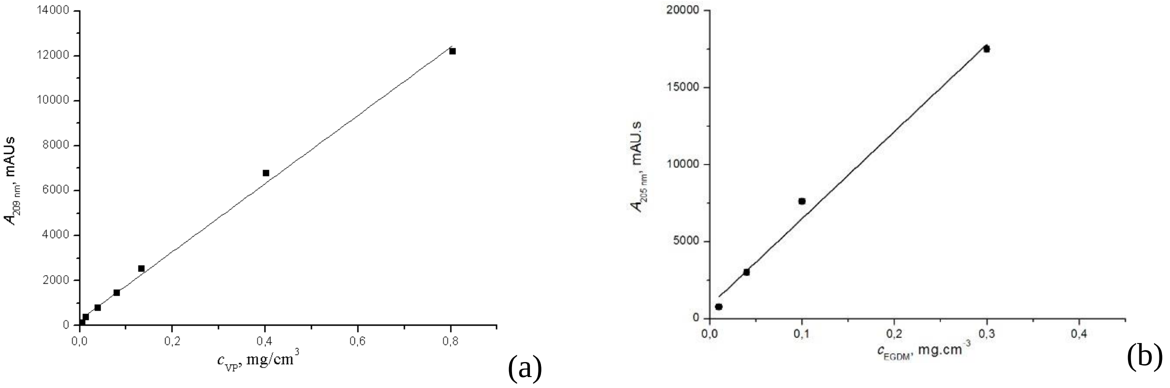


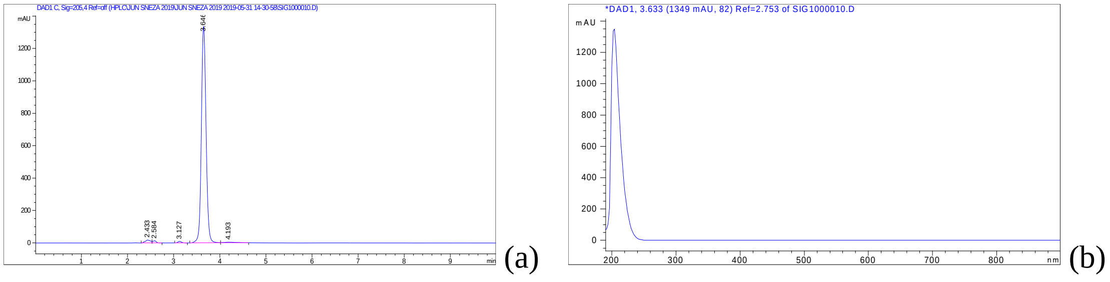
Figure 2. Calibration curve for: (a) 1-vinyl-2-pyrrolidone, (b) ethylene glycol dimethacrylate

Calibration curve for vinyl acetate

On the HPLC chromatogram of the standard solutions, vinyl acetate has a retention time R_t=3.666 min (Figure 3(a)). The wavelength at which the maximum absorption in the UV region for VA occurs is λ_{max}=205 nm. The UV spectrum of the VA standard is shown in Figure 3(b). For the constructed calibration curve of the comonomer VA (Figure 3(c)) in the linear range (for peak areas in the range of 300-7100 mAU·s, *i.e.*, in the range of 0.005-0.300 mg·cm^{-3}), the equation (4) applies:

$$A_{205nm} = 276{,}88 + 22856{,}46 \cdot c_{VA} \tag{4}$$

A_{205nm} is the peak area (mAU s) at λ_{max}= 205 nm, c_{VA} is the content of VA comonomer in mg·cm^{-3}, where the linear correlation coefficient was R^2=0.997, σ=155.299, LoD=0.0204 mg·cm^{-3}, and LoQ=0.0679 mg·cm^{-3}.



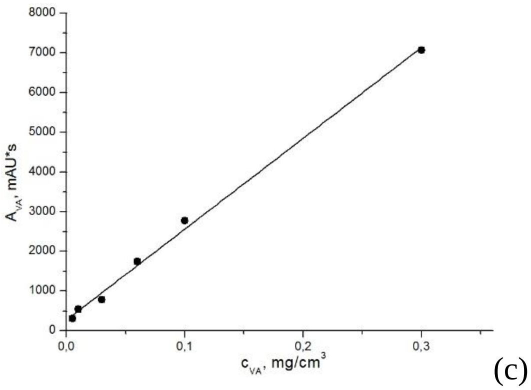


Figure 3. Vinyl acetate: (a) HPLC chromatogram, R_t=3.666 min, (b) UV spectrum, λ_{max}=205 nm, (c) calibration curve.

Unreacted amounts of comonomers and crosslinker after p(VP-VA) synthesis

Methanol extracts from polymerized p(VP-VA) copolymer hydrogels were analyzed using the HPLC method to determine the unreacted amount of monomer, comonomer, and crosslinker during polymerization. Data from the peaks integration of the analyzed methanol extracts and the obtained values of peaks area were in the range of the calibration curve. The unknown contents of VP, EGDM, and VA in the decanted methanol extracts were calculated based on the constructed calibration curves using equations (2), (3), and (4), respectively. The peaks of all reactants in the tested samples are sharp and symmetrical, and the chromatographic conditions were determined to allow clear separation and detection. The HPLC method for the determination of VP and VA in copovidone according to the Ph. Eur. monograph “Povidone” (Impurity A), comprising mobile phase water/acetonitrile 92/8 (%w/w), column temperature 40 °C, retention time: 9–11 min (vinyl acetate) and 12-14 min (vinylpyrrolidone) (Bühler, 2005). The fast methods using gel permeation chromatography (GPC) and reverse-phase high-pressure liquid chromatography (RP HPLC) techniques were used to separate and quantify residual VP monomer present in homopolymer p(VP) and a copolymer of VP and vinyl caprolactam (Senak et al., 2008). Fast GPC and RP HPLC analyses were quantitatively compared to industrial and European Pharmacopeia methodologies. The presented method was different from the published method in Eur Ph 5 and provided faster analysis at lower temperature (Bühler, 2005). The results of the unreacted quantities of VP, VA, and EGDM during p(VP-VA) synthesis were calculated in relation to their amount present in the reaction mixture at the beginning of reaction compared to the total xerogel mass (in $mg \cdot g^{-1}$), and to the initial amount present in the reaction mixture (in %), were shown in Tables 1 and 2, respectively.

Table 1. Unreacted amounts of 1-vinyl-2-pyrrolidone, vinyl acetate, and ethylene glycol dimethacrylate, calculated in relation to the total mass of p(VP-VA) xerogels (mg·g^{-1}), the peaks area values of the methanol extract samples and masses of xerogels samples.

p(VP-VA) sample	sample mass, g	unreacted reactants compared to the total xerogel mass, mg·g^{-1}					
		A_{VP}, mAU·s	VP	A_{VA}, mAU·s	VA	A_{EGDM}, mAU·s	EGDM
10/1.0	0.524	4409.1	14.682	978.2	1.757	486	0.379
10/1.5	0.529	2093.3	6.683	1370.7	2.714	523	0.404
10/2.0	0.531	2887.3	9.343	1104.2	2.045	1430	1.075
10/2.5	0.538	2617.8	8.322	1632.5	3.307	1391	1.032
10/3.0	0.541	1734.4	5.344	1221.2	2.291	578.9	0.435

Table 2. Unreacted amounts of 1-vinyl-2-pyrrolidone, vinyl acetate, and ethylene glycol dimethacrylate, compared to the initial amount present in the reaction mixture, %.

p(VP-VA) sample	unreacted reactants compared to the initial amount, %		
	VP	VA	EGDM
10/1.0	1.609	2.486	2.251
10/1.5	0.739	3.872	1.609
10/2.0	1.041	2.942	3.240
10/2.5	0.935	4.798	2.510
10/3.0	0.605	3.351	0.889

The obtained values of unreacted reactants compared to the total p(VP-VA) xerogel mass were in the range of 5.344-14.682 mg·g^{-1} for VP, 1.757-3.307 mg·g^{-1} for VA, and 0.379-1.075 mg·g^{-1} for EGDM (Table 1). The unreacted amounts in relation to the initial amount present in the reaction mixture were in the range of 0.605-1.609% for VP and 2.486-4.798% for VA, while for the EGDM in the range of 0.889-3.240% (Table 2). Although the presented results indicated that the free radical polymerization process did not lead to the complete conversion of the reactants, it was satisfactory for the presented study. Also, it is very important to perform removal of unreacted comonomers and crosslinker from the obtained hydrogels after polymerization, because of their toxicity, as the next phase of the production process. In this way, all the unreacted reactants are removed, so hydrogels can be safe for further application. Unreacted monomers contents generally calculated below 10% were found in various dental composite resins (Kwon et al., 2015). For an example, the maximum amount of residual monomer in polyvinyl pyrrolidone for pharmaceutical applications has been reported to be as high as 1% (IARC, 1979), which is in accordance with obtained results presented in Table 2 (0.605-1.609% for VP, 2.486-4.798% for VA, and 0.889-3.240% for EGDM). Also, it has been assumed that 1-vinyl-2-pyrrolidone is readily biodegradable (Bühler, 2005). According to the standard ISO 1567:1999, the maximal allowed amount of residual methyl methacrylate for heat-polymerized acrylates is 2.2%. In contrast, for cold-polymerized acrylates, it amounts to 4.5%, and the amounts of residual monomers in this study were in similar limits. Water-bath post-polymerization may be an efficient way to diminish potential acrylate toxicity by diffusing unpolymerized monomer in water, additional polymerization at places with active radicals, or hydrolysis to methacrylic acid (Jorge et al., 2006; Koda et al., 1990).

Structural characterization of p(VP-VA) hydrogels

In the FTIR spectrum of VP monomer molecule, the presence of a characteristic strong band with maximum absorption at 1705 cm^{-1} confirms the structure of a five-membered ring of cyclic amide, γ-lactam (Figure 4a). The presence of the vinyl group is confirmed by absorption bands with maxima at 3109 cm^{-1} and 1332 cm^{-1} of stretching vibrations asymmetric ν_{as}(=C-H) and symmetric ν_{s}(=C-H) of the vinyl group. Additional confirmation of the vinyl group presence is given by the bending vibrations in the plane, δ(=C-H), and out of the plane, γ(=C-H), at 1425 cm^{-1} and 981 cm^{-1}, respectively. Confirmation of the CH_2 group presence is given by the absorption bands at 2979 cm^{-1}, 2874 cm^{-1} and 1461 cm^{-1} originating from the stretching asymmetric, ν_{as}(CH), symmetric, ν_{s}(CH_2), and bending vibrations of the CH group, δ(CH), respectively. The principal characteristic of VP monomer is the presence of a lower intensity absorption band with a maximum at 1630 cm^{-1}, attributed to the stretching vibrations of the double C=C bond, ν(C=C) (D'Amelia et al., 2019). This FTIR spectrum was similar to previously published (Ilić-Stojanović and Eraković, 2019).

In the FTIR spectrum of vinyl acetate comonomer (Figure 3), a strong key band with a maximum at 1762 cm^{-1} is present and originates from the stretching ν(C=O) vibrations. The medium intensity absorption band with a maximum at 1648 cm^{-1} results from stretching vibrations of the double C=C bond, ν(C=C). The low-intensity absorption band with a maximum of 3095 cm^{-1} originates from asymmetric stretching vibrations of the vinyl group, ν_{as}(=CH), whose presence is also confirmed by the band from bending vibrations in the plane, δ(=CH), with a maximum at 1372 cm^{-1}. A characteristic, very strong band with an absorption maximum at 1219 cm^{-1} is attributed to the stretching ν_{as}(C-O) vibrations (D'Amelia et al., 2019).

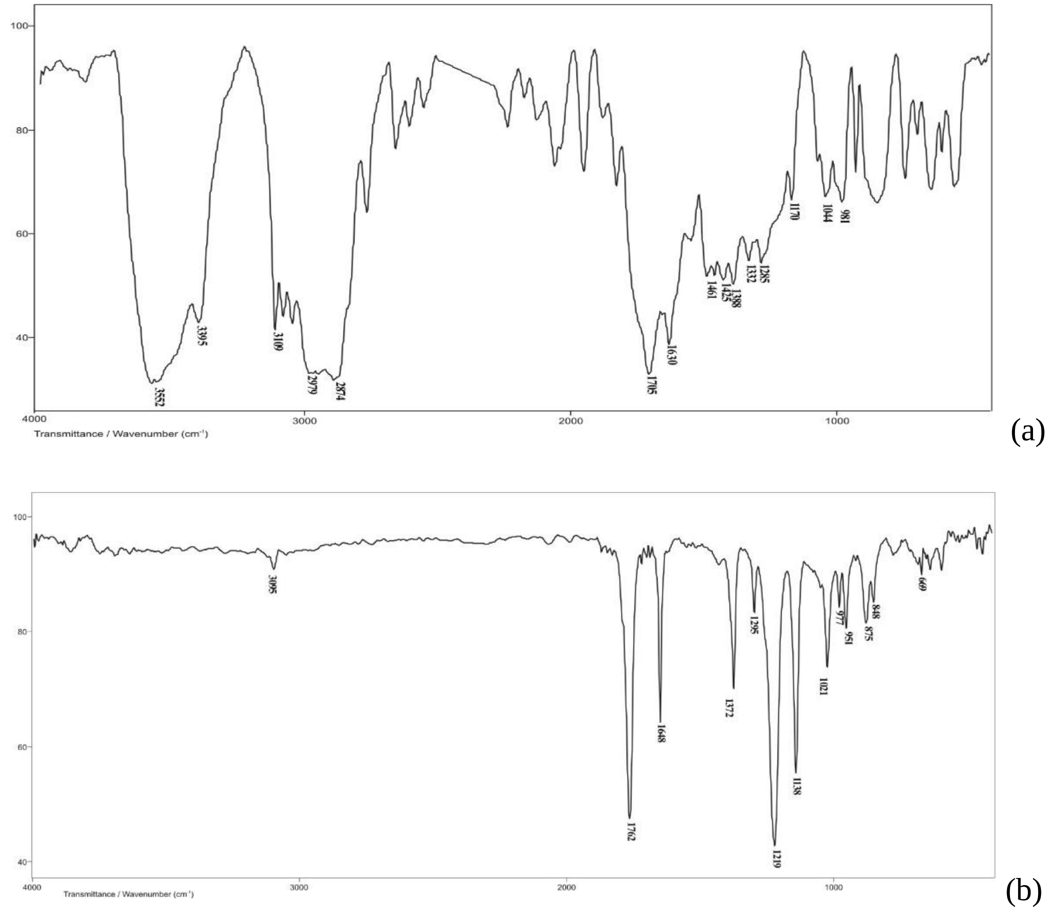


Figure 4. FTIR spectra of comonomers: (a) 1-vinyl-2-pyrrolidone, and (b) vinyl acetate.

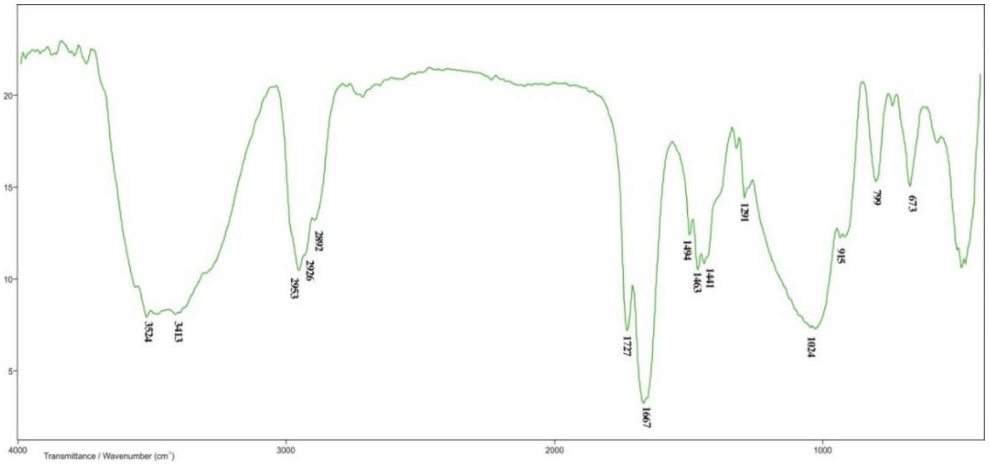


Figure 5. FTIR spectrum of p(VP-VA) hydrogel with 10 mol% of vinyl acetate comonomer.

The FTIR spectrum of the synthesized p(VP-VA) hydrogel with 10 mol% VA comonomer and 1.0 mol% of EGDM is shown in Figure 5. On the FTIR spectrum of the p(VP-VA) hydrogel, the absence of certain characteristic absorption bands present in the FTIR spectra of comonomers (VP and VA) and EGDM as crosslinker is observed which indicates that the structure of the new molecule was formed. There are no absorption bands from the stretching vibrations of the vinyl group (-CH=CH_2), ν(=CH), and bending vibrations in the plane, δ(=CH), and out of the plane, γ(=C-H), present in the spectra of comonomers and crosslinker (Figures 4 and 5). The absence of bands in the range of 1620-1640 cm^{-1} characteristic for stretching vibrations of the double C=C bond from VP and VA and EGDM, confirms that the copolymer structure was formed by double C=C bonds cleavage. Asymmetric stretching vibrations from the CH_3 and CH_2 groups, ν_{as}(C-H), give absorption band maxima at 2953, 2926, and 2892 cm^{-1}, respectively. Bending C-H vibrations in the plane, δ(C-H), from CH_3 and CH_2 groups show the maximum absorption at 1494 cm^{-1}. The sharp, intense band at 1727 cm^{-1} is a major characteristic of the p(VP-VA) hydrogel. It originates from the stretching vibrations of the C=O group, ν_{as}(C=O), from the remains of VP and VA molecules (D'Amelia et al., 2019). The maximum absorption of the characteristic band at 1291 cm^{-1} is attributed to the stretching vibrations of the C-O bond, ν_{as}(C-O) (D'Amelia et al., 2019; Ilić-Stojanović and Eraković, 2019).

The FTIR spectra of xerogel poly(1-vinyl-2-pyrrolidone-*co*-vinyl acetate) with 10 mol% vinyl acetate and 1.5, 2.0, 2.5 and 3.0 mol% of EGDM crosslinker show similarity. Structural analysis of the FTIR spectra of VP and VA comonomers, crosslinkers, and p(VP-VA) copolymers are in agreement with data from the literature (D'Amelia et al., 2019; Ilić-Stojanović and Eraković, 2019; Milosavljević, 2020; Sa'adun et al., 2014).

Thermosensitivity analysis

The sensitivity of the synthesized p(VP-VA) hydrogels with 10 mol% of VA and 1.0, 1.5, 2.0, 2.5 and 3.0 mol% of EGDM to changes in external temperature was examined by monitoring the change in the equilibrium swelling degree, α_e, with increasing fluid temperature from 25ºC to 80ºC in a fluid with pH value 6.0. Hydrogels were swollen to equilibrium at a temperature of 25ºC, the massess were measured, and the process of changing the hydrogels masess with increasing temperature was monitored. The temperature dependence of the swelling degree, α, on p(VP-VA) hydrogels was shown in Figure 6, and equilibrium swelling degrees, α_e, achieved at 25ºC and 80ºC, was presented in Table 3.

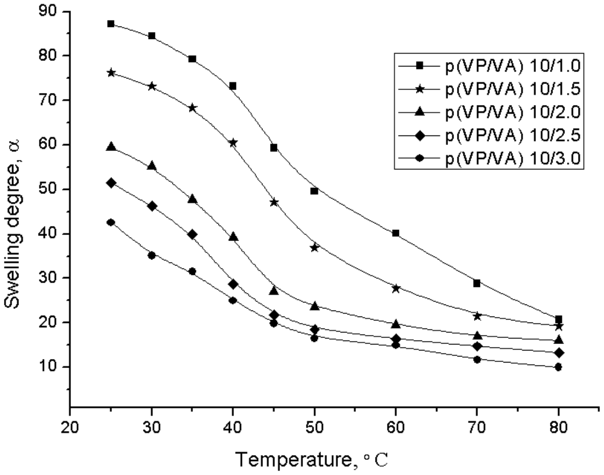


Figure 6. Temperature dependences of the swelling degree, α, p(VP-VA) hydrogels with 1.0, 1.5, 2.0, 2.5 and 3.0 mol% EGDM as crosslinker in fluid with pH value 6.0.

Table 3. Equilibrium swelling degree, α_e, of p(VP-VA) hydrogels at 25ºC and 80ºC.

p(VP-VA)	10/1.0	10/1.5	10/2.0	10/2.5	10/3.0
25°C	87.23	76.26	59.45	51.45	42.56
80°C	20.74	19.24	16.04	13.34	10.02

The swelling process of hydrogels p(VP-VA) was favored at a lower temperature (25°C) when the sample with 1.0 mol% EGDM was reached the highest swelling degree (1 g of a sample absorbed 87.23 g of water). Retention capacity continuously decreases with an increasing amount of EGDM in the hydrogel sample (1 g of a sample with 3 mol% absorbed 42.56 g of water). As the temperature increases, the swelling degree decreases for all samples of synthesized copolymers, and the achieved the most intense volume contraction in the temperature range from 40°C to 45°C (Figure 7). At the 80°C, the hydrogels dehydrated with the release of water from the polymer network, so that 1 g of the same sample with 1 mol% EGDM retained only 20.74 g of water. After hydrogel contraction, the swelling degree was asymptotically approached a constant value of approximately which was about 4 times lesser than at 25^0 C (Table 3). A proportional decrease in swelling capacity was noticed with an increase in crosslinking degree because these are polymer networks of higher density with less space between the network nodes. All synthesized hydrogels p(VP-VA) exhibit volume phase transition temperature (VPTT) in the range of 40–45 °C, which classifies them as negative thermo-sensitive (LCST). The sample with 3 mol% EGDM showed the smallest change in the swelling degree and in phase transition (from α_e=42.56 at 25ºC to α_e=10.02 at 80ºC). In the swollen state at lower temperatures, intermolecular hydrogen bonds between the free side keto groups of hydrogels (C=O) and ester C-O groups with -OH groups from water molecules are rewarded. When temperature increases above the LCST, the intermolecular hydrogen bonds break because long polymer C-C chains tend to reduce their surface area and lead to hydrogels dehydration (Ilić-Stojanović et al., 2017). Analyzed hydrogels show a thermosensitive reaction which is reflected in a significant decrease in the swelling with increasing external temperature. Based on the obtained results of sensitivity testing to changes in fluid temperature, crosslinked p(VP-VA) hydrogels with 10 mol% of VA and with 1.0, 1.5, 2.0, 2.5 and 3.0 mol% of EGDM can be classified as negative thermosensitive hydrogels having a lower critical solution temperature (LCST).

Conclusion

A series of poly(1-vinyl-2-pyrrolidone-co-vinyl acetate) copolymer hydrogels were successfully synthesized by the method of radical polymerization with thermal initiation in the presence of 2,2`-azobis(2-methylpropionitrile) as initiator. Analysis of FTIR spectra of xerogels p(VP-VA) showed that there are no bands of stretching and bending vibrations of the vinyl group, nor bands of stretching C=C vibrations (present in comonomers and crosslinker molecules), which indicates that polymerization and crosslinking of polymer chains was carried out by breaking double bonds. Quantities of residual reactants after hydrogels synthesis were in the in acceptable values, confirmed the successful synthesis, and they were removed from final products. Crosslinked p(VP-VA) hydrogels with 10 mol% of VA show sensitivity to temperature changes, and they are negatively thermosensitive, *i.e.*, they swell at lower temperatures and contract at higher temperatures. They could be applied as potential absorbents or carriers for various molecules.

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Conflict-of-Interest Statement

No potential conflict of interest was reported by the authors.

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Chemia Naissensis, Vol 4, Issue 2, RESEARCH ARTICLE, 49

Analize strukture i termoosetljivosti sintetisanih umreženih hidrogelova poli(1-vinil-2-pirolidon-ko-vinil acetata)

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SAŽETAK

U ovom radu opisan je postupak sinteze hemijski umreženih kopolimernih hidrogelova na bazi monomera 1-vinil-2-pirolidona i komonomera vinil-acetata, korišćenjem različitog sadržaja umreživača etilenglikoldimetakrilata (EGDM) metodom slobodnoradikalne polimerizacije sa termičkim iniciranjem. Sadržaj neproreagovanih reaktanata nakon sinteze hidrogelova poli(1-vinil-2-pirolidona-ko-vinil-acetata), p(VP-VA) ispitan je primenom metode tečne hromatografije visokog pritiska (HPLC). Strukturna karakterizacija dobijenih hidrogelova p(VP-VA), izvedena je primenom infracrvene spektroskopije sa Furijeovom transformacijom (FTIR). U ovom istraživanju analiziran je uticaj temperature i sadržaja umreživača na ponašanje prilikom bubrenja hidrogelova p(VP-VA). Neproreagovale količine 1-vinil-2-pirolidona (0,605-1,609%), vinil acetata (2,486-4,798%) i EGDM-a (0,889-3,240%), u odnosu na količinu prisutnu u reakcionoj smeši na početku reakcije, potvrdile su uspešnu konverziju u hidrogelove p(VP-VA). FTIR spektri su potvrdili da je do umrežavanja polimernih lanaca došlo raskidanjem dvostrukih veza vinil-grupa reaktanata. Dobijeni umreženi kopolimeri se mogu svrstati u klasu negativno-termoosetljivih hidrogelova, jer mogu da bubre i kada se zagrevaju prolaze kroz fazni prelaz iz nabubrelog stanja na 25°C u kontrahovano stanje na 80°C.

Ključne reči: hidrogel, hemijsko umrežavanje, 1-vinil-2-pirolidon, vinil-acetat, bubrenje, termoosetljivost

Chemia Naissensis, Vol 4, Issue 2, RESEARCH ARTICLE, 50-51

Analyses de la structure et des propriétés thermiques d'hydrogels de poly(1-vinyl-2-pyrrolidone-co-acétate de vinyle) réticulés synthétisés

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RÉSUMÉ

Cet article décrit le procédé de synthèse d'hydrogels de copolymères chimiquement réticulés à base de monomère 1-vinyl-2-pyrrolidone et de comonomère acétate de vinyle, utilisant la teneur variée en diméthacrylate d'éthylène glycol comme réticulant, par la méthode de polymérisation radicalaire avec initiation thermique. La teneur en réactifs n'ayant pas réagi après la synthèse d'hydrogels de poly (1-vinyl-2-pyrrolidone-co-vinyl-acétate), p (VP-VA), a été examinée à l'aide de la méthode de chromatographie liquide à haute pression (HPLC). La caractérisation de la structure des hydrogels p (VP-VA) obtenus a été réalisée à l'aide de la spectroscopie infrarouge à transformée de Fourier (FTIR). Dans cette étude, les influences de la teneur en réticulant et de la température sur le comportement de gonflement de p(VP-VA) ont été étudiées. Les quantités de comonomères n'ayant pas réagi et d'agent de réticulation, calculées par rapport à la quantité initiale présente dans le mélange réactionnel, ont confirmé leur conversion réussie en hydrogels p(VP-VA). Ces valeurs n'ayant pas réagi de 1-vinyl-2-pyrrolidone (dans la plage de 0,605 à 1,609 %), d'acétate de vinyle (dans la plage de 2,486 à 4,798%) et de diméthacrylate d'éthylène glycol (dans la plage de 0,889 à 3,240%) étaient dans des limites acceptables et ils ont été retirés des produits finaux. Les spectres FTIR ont été vérifiés que le processus de copolymérisation a été effectué et la réticulation chimique des chaînes polymères s'est produite en cassant les doubles liaisons des réactifs. Les copolymères réticulés obtenus pourraient être classés dans la classe des hydrogels thermosensibles négatifs, car ils peuvent gonfler et passer par une transition de phase lorsqu’ils sont chauffés de l’état gonflé à 25ºC à l’état contracté à 80ºC.

Mots-clés: hydrogel, réticulation chimique, 1-vinyl-2-pyrrolidone, acétate de vinyle, gonflement, thermosensibilité

Chemia Naissensis, Vol 4, Issue 2, RESEARCH ARTICLE, 52-53

Анализ структуры и термических свойств синтезированных перекрестно-сшитых поли(1-винил-2-пирролидон-со-винилацетат) гидрогелей

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АННОТАЦИЯ

В данной работе описан процесс синтеза химически перекрестно-сшитых сополимерных гидрогелей на основе мономера 1-винил-2-пирролидона и сомономера винилацетата с использованием различного содержания диметакрилата этиленгликоля в качестве сшивающего агента методом свободнорадикальной полимеризации с термическим инициированием. Содержание непрореагировавших реагентов после синтеза поли(1-винил-2-пирролидон-со-винилацетата), р(ВП-ВА), гидрогелей исследовали методом жидкостной хроматографии высокого давления (ВЭЖХ). Структурную характеристику полученных гидрогелей p(VP-VA) проводили с помощью инфракрасной спектроскопии с преобразованием Фурье (ИКПФ). В этом исследовании изучалось влияние содержания сшивающего агента и температуры на набухание p(VP-VA). Количества непрореагировавших сомономеров и сшивающего агента, рассчитанные по отношению к начальному количеству присутствующему в реакционной смеси, подтвердили их успешное превращение в гидрогели п(ВП-ВА). Эти непрореагировавшие количества 1-винил-2-пирролидона (в диапазоне 0,605–1,609%), винилацетата (в диапазоне 2,486–4,798%) и диметакрилата этиленгликоля (в диапазоне 0,889–3,240%) находились в допустимых пределах. и они были удалены из конечных продуктов. Спектры ИК-Фурье подтверждали, что процесс сополимеризации выполнялся, и происходило химическое сшивание полимерных цепей за счет разрыва двойных связей реагентов. Полученные сшитые сополимеры можно классифицировать к классу отрицательно-термочувствительных гидрогелей, так как они способны набухать и проходить фазовый переход при нагревании из набухшего состояния при 25°C в сжатое состояние при 80°C.

Ключевые слова: гидрогель, химическая сшивка, 1-винил-2-пирролидон, винилацетат, набухание, термочувствительность

Chemia Naissensis, Vol 4, Issue 2, RESEARCH ARTICLE, 54-55

Analysen der Struktur und der thermischen Eigenschaften synthetisierter vernetzter Poly(1-Vinyl-2-Pyrrolidon-Co-Vinylacetat)-Hydrogele

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ABSTRACT

Dieser Beitrag beschreibt das Verfahren zur Synthese chemisch vernetzter Copolymer-Hydrogele auf der Basis des Monomers 1-Vinyl-2-Pyrrolidon und des Komonomers Vinylacetat unter Verwendung des unterschiedlichen Gehalts an Ethylenglykoldimethacrylat als Vernetzer, nach der Methode der radikalischen Polymerisation mit thermischer Initiierung. Der Gehalt an nicht umgesetzten Reaktanten nach der Poly(1-Vinyl-2-Pyrrolidon-Co-Vinylacetat), p(VP-VA), Hydrogelsynthese wurde mittels Hochdruckflüssigkeitschromatographie (HPLC-Verfahren) untersucht. Die Strukturcharakterisierung der erhaltenen p(VP-VA)-Hydrogele wurde unter Verwendung der Fourier-Transformations-Infrarotspektroskopie (FTIR) durchgeführt. In dieser Studie wurden die Einflüsse von Vernetzergehalt und Temperatur auf das Quellverhalten von p(VP-VA) untersucht. Die Mengen an nicht umgesetzten Komonomeren und Vernetzern, berechnet in Bezug auf die Ausgangsmenge in der Reaktionsmischung, bestätigten ihre erfolgreiche Umwandlung in p(VP-VA)-Hydrogele. Diese nicht umgesetzten Werte von 1-Vinyl-2-Pyrrolidon (im Bereich von 0,605–1,609 %), Vinylacetat (im Bereich von 2,486–4,798 %) und Ethylenglycoldimethacrylat (im Bereich von 0,889–3,240 %) lagen innerhalb akzeptabler Grenzen und wurden aus den Endprodukten entfernt. Die FTIR-Spektren wurden bestätigt, dass der Kopolymerisationsprozess durchgeführt wurde, und eine chemische Vernetzung von Polymerketten erfolgte durch Aufbrechen von Doppelbindungen von den Reaktanten. Die erhaltenen vernetzten Kopolymere könnten in die Klasse der negativ-wärmeempfindlichen

Hydrogele eingeordnet werden, da sie quellen können und einen Phasenübergang durchlaufen, wenn sie vom gequollenen Zustand bei 25°C in den kontrahierten Zustand bei 80°C erhitzt werden.

Schlüsselwörter: Hydrogel, chemische Vernetzung, 1-Vinyl-2-Pyrrolidon, Vinylacetat, Quellung, Thermosensitivität

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Products of Prolonged Autoxidation of Simple Dihydric Phenols in the Presence of Copper(II) Ions - An Electron Spin Resonance Study

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ABSTRACT

Electron spin resonance (ESR) spectroscopy was used for characterizing the products obtained by prolonged autoxidation of simple dihydric phenols (hydroquinone, catechol, and 4-methylcatechol) in the presence of copper(II) ions. Room temperature ESR spectra revealed that both paramagnetic copper(II) ions and organic radicals are present in obtained autoxidation products similarly to the humic acid complexed copper(II) ions. The ratio of organic radical signal intensity to the copper(II) ion signal intensity suggests that the smallest amount of copper(II) ions is incorporated in the hydroquinone autoxidation product while the highest amount of copper(II) ions is incorporated in the autoxidation product of catechol. Satisfactory computer simulations of experimental ESR spectra were obtained by considering only one type of copper(II) ion binding site for hydroquinone autoxidation product and two distinct types of copper(II) ion binding sites for catechol and 4-methylcatechol autoxidation products. Parameters obtained by the computer simulation of ESR spectra indicated prevalent ionic bonding of copper(II) ions in polymeric matrices with tetrahedral distortion at copper(II) ion binding sites and negligible exchange interactions between them. Products obtained by the hydroquinone and catechol autoxidation have more similar characteristics in comparison to the product obtained by the 4-methylcatechol autoxidation where more expressed ionic bonding of copper(II) ions, and smaller tetrahedral distortion are present. Due to the dipolar interactions of oxygen-centered organic radicals in autoxidation products with paramagnetic copper(II) ions, their ESR linewidths are larger and *g*-values smaller in comparison to the values found in humic acids from various soil types.

Keywords: Hydroquinone, Catechol, 4-methylcatechol, Autoxidation, Copper(II) ion, ESR

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Introduction

Many simple phenolic compounds are highly susceptible to oxidation and autoxidation in aqueous solutions, and these processes are strongly influenced by the pH value (Friedman & Jürgens, 2000; Maier et al., 2018) and the presence of metal ions, especially transition metal ions (García et al., 1996; Nkhili et al., 2014; Rinaldi et al., 1995). Prolonged autoxidation (or O_2 oxidation) of polyhydric phenolic compounds or their corresponding quinones in highly alkaline aqueous solutions was employed for obtaining humic-acid like polymers (Barriquello et al., 2010; Doğan et al., 2013; Giannakopoulos et al., 2009; Litvin & Abi Njoh, 2020; Litvin et al., 2015) which have many potential applications. Current research on phenolic polymers covers various fields like antiviral activity (Bianculli et al., 2020), antioxidant properties (Alfieri et al., 2020), and possible uses as electrodes for advanced organic batteries (Patil et al., 2021), humic-like fertilizers (Cha et al., 2017) or for green coloration of textiles (Su et al., 2018). Polymers of phenolic compounds are usually obtained by their enzymatic oxidative polymerization (Alfieri et al., 2020; Dubey et al., 1998; Gamov et al., 2020; Kobayashi & Makino, 2009; Sun et al., 2013), but another viable alternative for their synthesis may be autoxidation in the presence of metal ions (McBride et al., 1988; Oess et al., 1999; Slikboer et al., 2015).

Of the many methods used to characterize natural and synthetic polymers, electron spin resonance (ESR, also known as electron paramagnetic resonance - EPR) spectroscopy proved to be especially useful (Naveed et al., 2018). ESR spectroscopy is also an indispensable tool in transition metal chemistry, given that many transition metal ions, like copper(II) ion, are paramagnetic (Goodman & Raynor, 1970). Having the aforementioned in mind, we applied ESR spectroscopy for the characterization of the products of prolonged autoxidation of frequently used dihydric phenols (hydroquinone, catechol, and 4-methylcatechol) in the presence of copper(II) ions since these results can give valuable information on both copper(II) ion binding environment and organic radical(s) in obtained polymer matrices. Chemical structures of dihydric phenols used in this study are given in Figure 1.

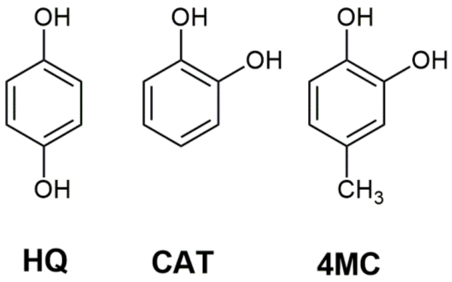

Figure 1. Chemical structures of hydroquinone (HQ), catechol (CAT), and 4-methylcatechol (4MC).

Experimental

Materials

Hydroquinone (HQ), catechol (CAT), and 4-methylcatechol (4MC) were obtained from Sigma-Aldrich (USA), and copper(II) chloride dihydrate ($CuCl_2 \times 2H_2O$) was obtained from Merck (Germany). Buffer compounds, sodium hydroxide, and hydrochloric acid were obtained from a local supplier (Centrochem, Serbia). All the chemicals were of analytical grade and were used without further purification. Demineralized water was used for preparing all aqueous solutions.

Sample preparation

The method used for samples prepared in this work is analogous to the methods used for obtaining phenolic polymers without a catalyst or additional oxidant (Barriquello et al., 2010; Doğan et al., 2013; Giannakopoulos et al., 2009). One gram of a particular phenolic compound (9.1 mmol of hydroquinone and catechol; 8.1 mmol of 4-methylcatechol) was dissolved in 28 mL of phosphate buffer (pH 7.4) in a round-bottom flask. The exact mass of $CuCl_2 \times 2H_2O$ necessary to make the phenol and Cu(II) ion molar ratio of 10 : 1 in solution (0.155 g for hydroquinone and catechol; 0.137 g for 4-methylcatechol) was ultrasonically dispersed in about 2 mL of phosphate buffer and added to stirring phenol solution in small portions. After thorough mixing, a pH value was accurately adjusted to 7.4 by the dropwise addition of 1M NaOH solution. Reaction mixtures were continuously stirred in an open flask for 96 hours at room temperature (22±2 °C) without exposure to direct sunlight. After 96 hours, the pH value was adjusted to ≈2 by adding 1M HCl solution, and mixtures were left for 72 hours without stirring at room temperature for the reaction product to precipitate. Reaction mixtures were then centrifuged at 4000 rpm for 15 minutes and filtered under vacuum. Collected precipitates were rinsed, first with 50 mL of demineralized water and then 50 mL of 0.01 M HCl solution. Precipitates were dried for 48 hours at 40 °C before ESR measurements.

ESR measurements and spectra handling

An X-band (9.85 GHz nominal frequency) EMX ESR spectrometer (Bruker, Germany) was used for recording ESR spectra of powder samples placed in 2 mm i.d. quartz tube cells at room temperature. Solid DPPH (2,2-diphenyl-1-picrylhydrazyl) was used as a standard (g = 2.0036) for g-value determination of organic radicals, while g-values of copper(II) ion binding sites were determined by computer simulations of ESR spectra. ESR spectra handling and computer simulations were performed on a personal computer by using WinEPR and SimFonia programs (Bruker, Germany).

Results and Discussion

ESR spectra of products of prolonged autoxidation of hydroquinone, catechol, and 4-methylcatechol in the presence of copper(II) ions are given in Figure 2.

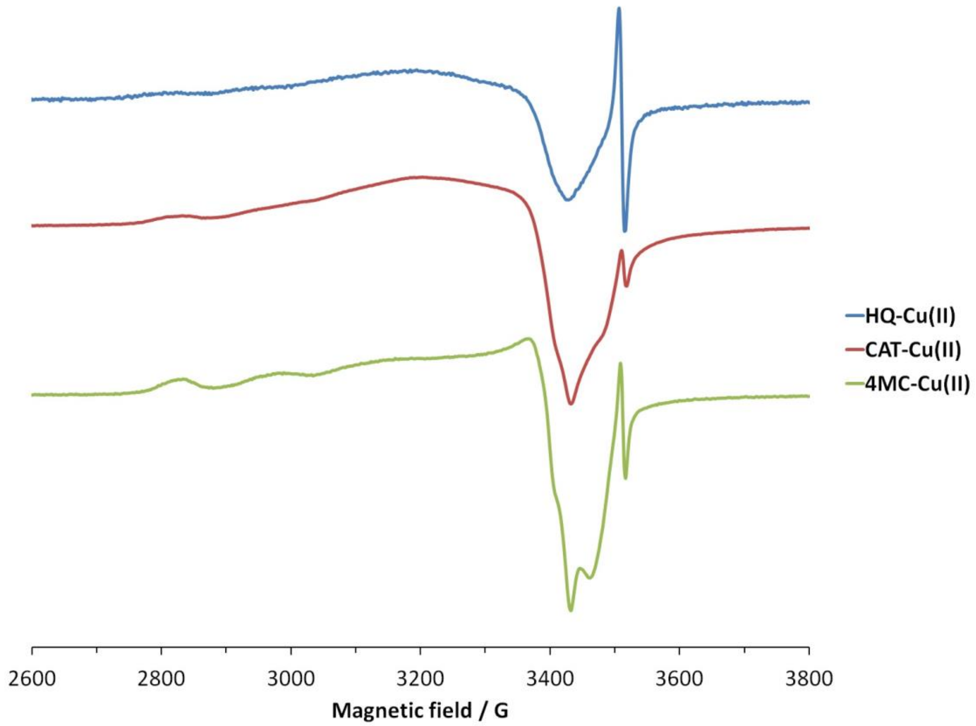

Figure 2. ESR spectra of products of prolonged autoxidation of hydroquinone, catechol, and 4-methylcatechol in the presence of copper(II) ions. Instrumental parameters: attenuation - 25 dB

(microwave power - 0.585 mW), modulation frequency - 100 kHz, modulation amplitude - 2 G, time constant - 0.64 ms, conversion time - 81.92 ms, center field - 3500 G, scan width - 2000 G.

A common characteristic of the ESR spectra shown in Figure 2 is the presence of two distinct spectral contributions: 1) broad resonance lines originating from the presence of copper(II) ions, and 2) one sharp resonance line at higher values of magnetic field originating from the presence of organic radical(s). The overall appearance of these spectra, *i.e.*, the ratio of organic radical signal intensity to the copper(II) ion signal intensity, suggests that the smallest amount of copper(II) ions is incorporated in the hydroquinone autoxidation product while the highest amount of copper(II) ions is incorporated in the autoxidation product of catechol.

Spectral features originating from the presence of copper(II) ions show resemblance to the ESR spectra reported in the literature for copper(II) ions in polymer matrices like polysaccharides (Bartkowiak et al., 1998; Hoffmann et al., 2008; Mitić et al., 2011), starch (Łabanowska et al., 2008) and lignin (Hoffmann et al., 2008; Merdy et al., 2002), where copper(II) ions coordination with only O-donor ligands may be expected. Similar ESR spectra were also reported for copper(II) ions adsorbed on humic acids (Bryukhovetskaya et al., 2016; Cheshire et al., 1977) and synthetic humic acid-like phenolic polymers (Barriquello et al., 2010). The absence of low-field spectral lines in the ESR spectra recorded with enlarged field scale (4000 G scan width, starting from 0 G) eliminates the possibility that binuclear copper(II) binding sites (S = 1) are present in analyzed samples (Etcheverry et al., 2012; Kozlevčar & Šegedin, 2008). Therefore, we assume that our samples contain mononuclear copper(II) centers (d^9, S = 1/2), and the general appearance of ESR spectra points towards their axial symmetry with the unpaired electron being placed in a $d_{x^2-y^2}$ ground state. Principal parameters that characterize such bonding sites are parallel and perpendicular hyperfine coupling constants ($A_\parallel$ and $A_\perp$, respectively) originating from the interaction of the unpaired electron with the magnetic moments of ^{63}Cu and ^{65}Cu nuclei (I = 3/2) and parallel and perpendicular g-values ($g_\parallel$ and $g_\perp$ respectively). These parameters can be extracted by either measuring directly from the experimental ESR spectra or by computer simulation of spectra. They then may be used to calculate many values useful for the characterization of copper(II) ion binding in systems under study.

The geometrical parameter G is a measure of the exchange interaction between the copper centers (Procter et al., 1968) and is calculated by the following equation:

$$\mathrm{G} = \frac{g_{\parallel} - g_e}{g_{\perp} - g_e}$$

where g_e is the g-value of the free electron (g_e = 2.0023). Exchange interaction is negligible if G > 4, and considerable exchange interaction is indicated if G < 4.

The in-plane σ bonding parameter α^2 is a covalency parameter (Kivelson & Neiman, 1961) and can be calculated by the following equation:

$$\alpha^2 = \frac{|\mathrm{A}_{\parallel}|}{\mathrm{P}} + \left(g_{\parallel} - g_e\right) + \frac{3}{7}\left(g_{\perp} - g_e\right) + 0.04$$

where P presents the direct dipolar term with the value of 360×10^{-4} cm^{-1} (McGarvey, 1967). The value of α^2 is connected with the degree of unpaired electron localization in the copper(II) ion $d_{x^2-y^2}$ orbital and if $\alpha^2 = 1$ the ligand-copper(II) ion bond would be completely ionic, while if $\alpha^2 = 0.5$ the bond would be completely covalent.

The value of *f*, an empirical index of tetrahedral distortion of copper(II) binding sites (Sakaguchi et al., 1979), may be conveniently calculated as:

$$f = \frac{g_{\parallel}}{|\mathrm{A}_{\parallel}|}$$

For square-planar structures, *f* values range from ~105 to 135 cm, but values higher than 150 cm are observed for tetrahedrally distorted copper(II) complexes.

Values of parallel and perpendicular hyperfine coupling constants and parallel and perpendicular *g*-values were obtained by the computer simulation of experimental ESR spectra. For example, experimental and computer-simulated ESR spectra of the product of prolonged autoxidation of 4-methylcatechol in the presence of copper(II) ions are shown in Figure 3.

As can be seen, the match between the experimental and simulated ESR spectrum in Figure 3 is not so good in the ~3000 to 3400 G range where high-field parallel and low-field perpendicular spectral components overlap. The same discrepancy is observed between the experimental and simulated ESR spectra of hydroquinone and catechol autoxidation products. The reason for that is the distribution of molecular binding parameters in amorphous and polymeric materials, which restricts the quality of computer simulations since simulation algorithms are usually adjusted for typical polycrystalline solids (Hoffmann et al., 2008). Satisfactory spectral simulation in the case of hydroquinone autoxidation product was obtained by considering one type of copper(II) ion binding site, but for catechol and 4-methylcatechol autoxidation products, reasonably good results were obtained only if the presence of two distinct types of copper(II) ion binding sites (species) was assumed. This may be the consequence of presumably the lowest amount of copper(II) ions being incorporated in the hydroquinone autoxidation product.

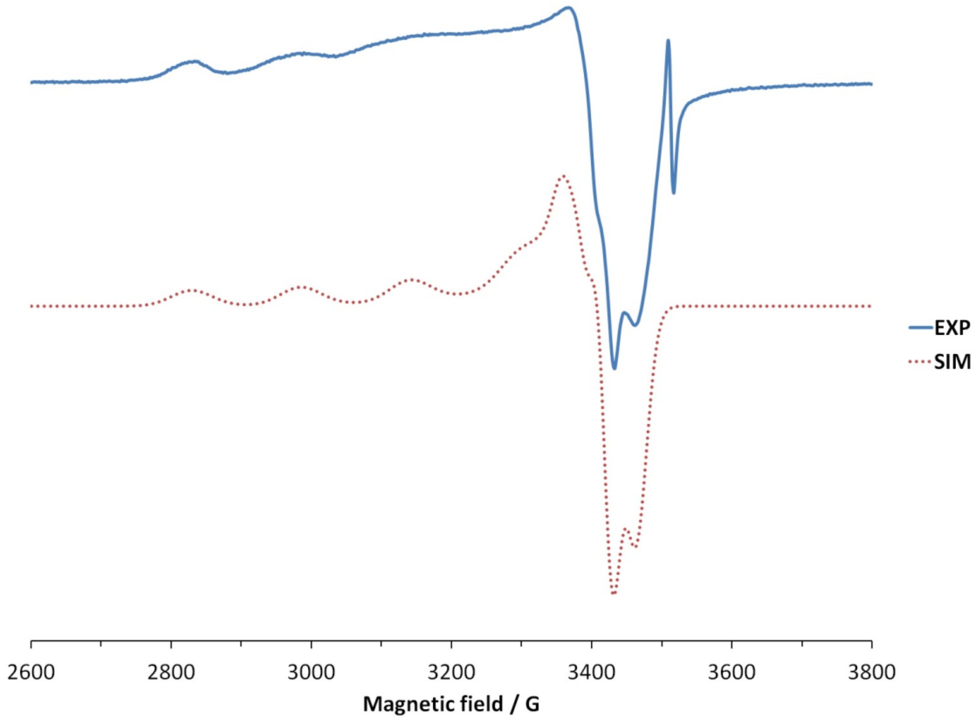

Figure 3. Experimental and computer-simulated ESR spectra of the product of prolonged autoxidation of 4-methylcatechol in the presence of copper(II) ions.

The ESR parameters determined by spectral simulation, together with the calculated values of G, α^2, and *f*, for the samples analyzed in this work are given in Table 1.

Table 1. ESR spectral parameters of copper(II) ions in analyzed samples.

Sample	Species (fraction)	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}\times10^4$ (cm^{-1})	$A_{\perp}\times10^4$ (cm^{-1})	G	α^2	*f* (cm)
HQ-Cu(II)	/	2.341	2.080	145.0	15.0	4.36	0.815	161.45
CAT-Cu(II)	A (0.60)	2.350	2.078	140.0	12.0	4.59	0.809	167.86
	B (0.40)	2.320	2.050	150.0	15.0	6.66	0.795	154.67

4MC-Cu(II)	A (0.41)	2.305	2.085	167.5	8.5	3.66	0.843	137.61
	B (0.59)	2.300	2.064	167.0	7.7	4.82	0.828	137.72

Values of α^2 for our samples indicate predominant ionic bonding of copper(II) ions. The α^2 values for the hydroquinone and catechol autoxidation products are mutually similar and comparable to the values reported for copper(II)-pullulan complexes synthesized at high pH values (Mitić et al., 2011) but are notably smaller than values reported for copper(II) ions introduced as a paramagnetic probe into starch (Łabanowska et al., 2008). Both species found in the autoxidation product of 4-methylcatechol have higher α^2 values, *i.e.*, more expressed copper(II) ionic bonding, than autoxidation products of hydroquinone and catechol. At the same time, according to the *f* values, these two species seem to have the smallest degree of tetrahedral distortion. Except for the specie A in autoxidation product of 4-methylcatechol, the exchange interaction between the copper(II) centers in our samples may be considered negligible.

ESR signals of organic radicals in our phenolic autoxidation products appear as well separated single lines at the high-field end of the ESR spectra shown in Figure 2. Unlike well resolved ESR spectra of organic radicals obtained during the initial steps of autoxidation of catechol in the presence of Al(III) (McBride & Sikora, 1990) or Mg(II) ions (Nikolić et al., 2019), ESR spectra of organic radicals in phenolic polymers consist of one single broad line (Barriquello et al., 2010; Giannakopoulos et al., 2009; Litvin et al., 2015) with spectral parameters similar to natural humic acids (Watanabe et al., 2005; and references therein). To obtain more accurate values for linewidths and g-values of organic radicals, ESR spectra of investigated samples were recorded under the same conditions listed in the legend of Figure 1, except that scan width was decreased to 200 G to increase spectral resolution. ESR spectral parameters of organic radicals in samples studied in this work are given in Table 2.

Table 2. ESR spectral parameters of organic radicals in investigated samples

Sample	Linewidth / G	*g*-value
HQ-Cu(II)	8.1	2.0027
CAT-Cu(II)	6.6	2.0014
4MC-Cu(II)	7.1	2.0028

Linewidths found in our samples somewhat exceed the usual range of linewidths characteristic for ESR signals of humic acids from a wide range of soil types (Watanabe et al., 2005), and this is probably the consequence of dipolar interactions of oxygen-centered radical species with paramagnetic copper(II) ion binding sites present in polymer matrices. On the other hand, *g*-values for our samples are lower than the values for both natural humic acids and synthesized humic acid-like polymers (Barriquello et al., 2010). The characterization of organic radical ESR signal was not performed for the samples obtained by the polymerization of simple phenols in the presence of copper(II) ions (Oess et al., 1999) or by the adsorption of copper(II) ions on humic acids (Bryukhovetskaya et al., 2016) and synthetic humic acid-like polymers (Barriquello et al., 2010) but there are literature data that even addition of diamagnetic Pb(II) ions to humic acids causes an appreciable decrease of their radicals *g*-values (Giannakopoulos et al., 2005). It is also worth noting that the smallest *g*-value in our samples was measured for the autoxidation product of catechol which was supposed to have the highest amount of copper(II) ion incorporated.

Conclusion

Electron spin resonance (ESR) study of products obtained by the prolonged autoxidation of simple dihydric phenols (hydroquinone, catechol, and 4-methylcatechol) in the presence of copper(II) ions revealed that they contain both paramagnetic copper(II) ions and organic radicals, similarly to the humic acid complexed copper(II) ions. According to the ratio of organic radical ESR signal intensity to the copper(II) ion ESR signal intensity, we concluded that the smallest amount of copper(II) ions was incorporated in the hydroquinone autoxidation product while the highest amount of copper(II) ions was incorporated in the autoxidation product of catechol. Parallel and perpendicular hyperfine splitting constants and *g*-values for copper(II) ions were obtained by computer simulations of experimental ESR spectra under the assumption that only one type of copper(II) ion binding site with axial symmetry exists in hydroquinone autoxidation product while two distinct types of copper(II) ion binding sites with axial symmetry exist in catechol and 4-methylcatechol autoxidation products. Obtained parameters allowed the calculation of values used for estimating exchange interactions between copper(II) ions, the degree of ionic binding of copper(II) ions in polymer matrices, and tetrahedral distortion of copper(II) ion bonding sites. Prevalent ionic bonding of copper(II) ions in polymeric matrices with tetrahedral distortion at copper(II) ion binding sites and negligible exchange interactions between them was found for all three investigated samples. Products obtained by the hydroquinone and catechol autoxidation have more similar characteristics compared to the product obtained by the 4-methylcatechol autoxidation where more expressed ionic bonding of copper(II) ions and smaller tetrahedral distortion are present. Oxygen-centered organic radicals in autoxidation products have larger ESR linewidths and smaller g-values in comparison to the values found in humic acids from various soil types due to the dipolar interactions with paramagnetic copper(II) ions.

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Conflict-of-Interest Statement

None.

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Proizvodi produžene autooksidacije jednostavnih dvohidroksilnih fenola u prisustvu bakar(II) jona - Ispitivanje elektronskom spinskom rezonancom

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SAŽETAK

Elektronska spinska rezonanca (ESR) iskorišćena je za karakterizaciju proizvoda dobijenih produženom autooksidacijom jednostavnih dvohidroksilnih fenola (hidrohinona, katehola i 4-metilkatehola) u prisustvu bakar(II) jona. ESR spektri snimljeni na sobnoj temperaturi pokazali su prisustvo paramagnetnih bakar(II) jona i organskih radikala u dobijenim proizvodima autooksidacije, slično kao kod kompleksa huminskih kiselina sa bakar(II) jonima. Odnos intenziteta signala organskih radikala i bakar(II) jona upućuje na to da je najmanja količina bakar(II) jona ugrađena u proizvod autooksidacije hidrohinona, dok je najveća količina bakar(II) jona ugrađena u proizvod autooksidacije katehola. Zadovoljavajuće kompjuterske simulacije eksperimentalnih ESR spektara dobijene su pod pretpostavkom da se kod proizvoda autooksidacije hidrohinona javlja samo jedan tip vezujućeg mesta za bakar(II) jone dok se kod proizvoda autooksidacije katehola i 4-metilkatehola javljaju dva različita tipa vezujućih mesta za bakar(II) jone. Parametri dobijeni kompjuterskom simulacijom ESR spektara ukazuju na pretežno jonsko vezivanje bakar(II) jona u okviru polimerne matrice sa tetraedarskom distorzijom vezujućih mesta i zanemarljivom interakcijom izmene između njih. Proizvodi dobijeni autooksidacijom hidrohinona i katehola imaju međusobno slične osobine, dok je kod proizvoda autooksidacije 4-metilkatehola izraženiji jonski karakter veze sa manjom tetraedarskom distorzijom vezujućih mesta za bakar(II) jone. Zbog dipolarnih interakcija kiseonično centriranih organskih radikala sa paramagnetnim bakar(II) jonima, njihove širine ESR signala veće su a *g*-vrednosti manje u poređenju sa odgovarajućim vrednostima kod huminskih kiselina iz različitih tipova zemljišta.

Ključne reči: hidrohinon, katehol, 4-metilkatecol, autooksidacija, bakar(II) jon, ESR

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Produits d'auto-oxydation prolongée de phénols dihydriques simples en présence d'ions cuivre (II) - Une étude de résonance de spin électronique

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RÉSUMÉ

La spectroscopie par résonance de spin électronique (ESR) a été utilisée pour caractériser les produits obtenus par auto-oxydation prolongée de phénols dihydriques simples (hydroquinone, catéchol et 4-méthylcatéchol) en présence d'ions cuivre(II). Les spectres ESR à température ambiante ont révélé que les ions de cuivre (II) paramagnétiques et les radicaux organiques sont présents dans les produits d'auto-oxydation obtenus de la même manière que les ions de cuivre (II) complexés par l'acide humique. Le rapport de l'intensité du signal des radicaux organiques à l'intensité du signal des ions cuivre (II) suggère que la plus petite quantité d'ions cuivre (II) est incorporée dans le produit d'auto-oxydation de l'hydroquinone tandis que la plus grande quantité d'ions cuivre (II) est incorporée dans le produit d'auto-oxydation du catéchol. Des simulations informatiques satisfaisantes des spectres ESR expérimentaux ont été obtenues en considérant un seul type de site de liaison des ions cuivre (II) pour le produit d'auto-oxydation de l'hydroquinone et deux types distincts de site de liaison des ions cuivre (II) pour les produits d'auto-oxydation du catéchol et du 4-méthylcatéchol. Les paramètres obtenus par la simulation informatique des spectres ESR ont indiqué une liaison ionique prédominante des ions cuivre (II) dans les matrices polymères avec une distorsion tétraédrique au niveau des sites de liaison des ions cuivre (II) et des interactions d'échange négligeables entre eux. Les produits obtenus par l'auto-oxydation de l'hydroquinone et du catéchol ont des caractéristiques plus similaires par rapport au produit obtenu par l'auto-oxydation du 4-méthylcatéchol où une liaison ionique plus exprimée des ions cuivre (II) et une distorsion tétraédrique plus faible sont présentes. En raison des interactions dipolaires des radicaux organiques centrés sur l'oxygène dans les produits d'auto-oxydation avec des ions de cuivre (II) paramagnétiques, leurs largeurs de raie

ESR sont plus grandes et leurs valeurs g plus petites par rapport aux valeurs trouvées dans les acides humiques de divers types de sol.

Mots-clés: Hydroquinone, Catéchol, 4-méthylcatéchol, Auto-oxydation, Ion Cuivre(II), ESR

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Продукты продолжительного автоокисления простых двухатомных фенолов в присутствии ионов меди(II) - исследование электронным спиновим резонансом

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АННОТАЦИЯ

Спектроскопией электронного спинового резонанса (ЭПР) охарактеризовали продукты, полученные при продолжительном автоокислении простых двугидроксильных фенолов (гидрохинона, катехина и 4-метилкатехина) в присутствии ионов меди(II). Спектры ЭПР при комнатной температуре показали, что в полученных продуктах автоокисления присутствуют как парамагнитные ионы меди(II), так и органические радикалы, аналогично ионам меди(II) в комплексах гуминовых кислот. Отношение интенсивности сигнала органических радикалов к интенсивности сигнала ионов меди (II) предполагает, что наименьшее количество ионов меди (II) включается в продукт автоокисления гидрохинона, в то время как наибольшее количество ионов меди (II) включается в продукт автоокисления катехола. Удовлетворительное компьютерное моделирование экспериментальных спектров ЭПР было получено при рассмотрении только одного типа центров связывания ионов меди (II) для продукта автоокисления гидрохинона и двух различных типов центров связывания ионов меди (II) для продуктов автоокисления катехола и 4-метилкатехина. Параметры, полученные при компьютерном моделировании спектров ЭПР, свидетельствовали о преобладании ионной связи ионов меди(II) в полимерных матрицах с тетраэдрическим искажением в местах связывания ионов меди(II) и незначительных обменных взаимодействиях между ними. Продукты, полученные автоокислением гидрохинона и катехина, имеют более близкие характеристики по сравнению с продуктом, полученным автоокислением 4-метилкатехина, где присутствует более выраженная ионная связь ионов меди(II) и меньшее тетраэдрическое искажение. Из-за диполярного взаимодействия кислородцентрированных органических радикалов в продуктах автоокисления с парамагнитными ионами меди(II) ширина их линий ЭПР больше, а значения g меньше по сравнению со значениями, обнаруженными в гуминовых кислотах из различных типов почв.

Ключевые слова: гидрохинон, катехол, 4-метилкатехин, автоокисление, ион меди(II), ЭСР

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Produkte der verlängerten Autoxidation einfacher zweiwertiger Phenole in Gegenwart von Kupfer(II)-Ionen – eine Elektronenspinresonanzstudie

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ABSTRACT

Die Elektronenspinresonanz (ESR)-Spektroskopie wurde zur Charakterisierung der Produkte verwendet, die durch längere Autoxidation von einfachen zweiwertigen Phenolen (Hydrochinon, Brenzcatechin und 4-Methylbrenzcatechin) in Gegenwart von Kupfer(II)-Ionen erhalten wurden. Die ESR-Spektren bei Raumtemperatur zeigten, dass sowohl paramagnetische Kupfer(II)-Ionen als auch organische Radikale in den erhaltenen Autoxidationsprodukten vorhanden sind, ähnlich wie die mit Huminsäure komplexierten Kupfer(II)-Ionen. Das Verhältnis der Signalintensität organischer Radikale zur Signalintensität der Kupfer(II)-Ionen legt nahe, dass die kleinste Menge an Kupfer(II)-Ionen in das Autoxidationsprodukt von Hydrochinon eingebaut wird, während die höchste Menge an Kupfer(II)-Ionen in das Autoxidationsprodukt von Catechol eingebaut wird. Zufriedenstellende Computersimulationen von experimentellen ESR-Spektren wurden erhalten, indem nur eine Art von Kupfer(II)-Ionen-Bindungsstelle für das Hydrochinon-Autoxidationsprodukt und zwei unterschiedliche Arten von Kupfer(II)-Ionen-Bindungsstellen für Catechol- und 4-Methylcatechin-Autoxidationsprodukte betrachtet wurden. Die Parameter, die durch die Computersimulation von ESR-Spektren erhalten wurden, zeigten eine vorherrschende ionische Bindung von Kupfer(II)-Ionen in Polymermatrices mit tetraedrischer Verzerrung an Kupfer(II)-Ionen-Bindungsstellen und vernachlässigbaren Austauschwechselwirkungen zwischen ihnen. Produkte, die durch Hydrochinon- und Brenzcatechin-Autooxidation erhalten werden, haben ähnlichere Eigenschaften im Vergleich zu dem Produkt, das durch die 4-Methylbrenzcatechin-Autoxidation erhalten wird, wo stärker ausgeprägte ionische Bindungen von Kupfer(II)-Ionen und eine geringere tetraedrische Verzerrung vorhanden sind. Aufgrund der dipolaren Wechselwirkungen sauerstoffzentrierter organischer Radikale in Autoxidationsprodukten mit paramagnetischen Kupfer(II)-Ionen sind ihre ESR-Linienbreiten größer und ihre g-Werte kleiner im Vergleich zu den Werten, die in Huminsäuren aus verschiedenen Bodentypen gefunden werden.

Schlüsselwörter: Hydrochinon, Brenzcatechin, 4-Methylbrenzcatechin, Autoxidation, Kupfer(II)-Ion, ESR

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Extraction of selected elements from commercial fertilizers

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ABSTRACT

Two commercial fertilizers preparation methods for ICP OES analysis of were used for two different fertilizers types: urea and NPK (mineral fertilizer containing nutrients N, P, and K), as organic and inorganic fertilizers, respectively. The aim of our research was the comparison of two methods for the preparation of samples, the determination of the contents of elements, and the comparison of obtained results with the maximum allowed concentrations. The first preparation method consists of digestion with 18.5% HCl, and the other method is the digestion with *aqua regia*. Regarding the extraction of some secondary nutrients and micronutrients (Ca, Mg, Fe, Mn, Cu, Zn, and B) from NPK fertilizer, better extraction was achieved using the first method for B, Ca, and Mg, while the other method was better for Fe, Mn, Cu, and Zn. On the basis of the results of the analysis, the digestion with *aqua regia* is suitable for almost all investigated 3d metals from both samples, except for NPK, for which the degree of the extraction is somewhat higher. Determined concentrations of some toxic elements (As, Cr, Cd, Ni, and Pb) do not cross the maximum allowable concentrations (MAC).

Keywords: urea, NPK fertilizer, elements, ICP OES

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Introduction

Agricultural productivity can be enhanced using fertilizers because they contain nutrients, but they can also have toxic elements responsible for soil contamination (Li et al., 2013; Lima et al., 2015; Machado et al., 2017; Viso and Zachariadis, 2018). In general, fertilizers can be divided into natural and synthetic (inorganic, *e.g.*, NPK, and organic, *e.g.*, urea). Primary nutrients are N, P, and K; secondary nutrients are Ca, Mg, Na, and S; micronutrients are B, Co, Cu, Fe, Mn, Mo, and Zn; and trace elements are As, Cd, Cr, Ba, Ni, Se, V, U, Th and Pb (Otero et al., 2005).

The optimal quantity of NPK varies with soil types and climatic conditions (Muimba-Kankolongo, 2018). Urea contains 46% N, and it is the most concentrated solid nitrogen fertilizer, which in the soil goes to ammonium carbonate, and, therefore, can change local pH (Finch et al., 2014).

Determination of toxic elements in various fertilizers was performed using a couple of techniques based either on 1) Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), 2) The High-Resolution Continuous Source Flame Absorption Spectroscopy (HR-CS-FAAS) (Bechlin et al., 2014; Borges et al., 2011; Borges et al., 2015; De Morais et al., 2017; De Oliveira Souza et al., 2015); or 3) Cold Vapor Atomic Absorption Spectroscopy (CV-AAS) for Hg (Zhao and Wang, 2010). Regarding nutrients in fertilizers, their concentrations were determined using LIBS (Laser-Induced Breakdown Spectroscopy) (Andrade and Rodrigues Pereira-Filho, 2016; Nicolodelli et al., 2016; Nunes et al., 2014), and Total X-Ray Fluorescence (TXRF) (Resende and Nascentes, 2016). Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES) is fast, and it can be used for the determination of both nutrients and toxic metals in fertilizers, with minimal interference (Bartos, 2014; Kane and Hall, 2006; Kimura, 2016; Nziguheba and Smolders, 2008; Rui et al., 2012).

This study presents the results of the determination of selected elements using ICP OES in two fertilizers: inorganic (NPK) and organic (urea), using two different methods to prepare samples.

Experimental

The determination of the concentrations of selected elements in the samples of commercial fertilizers was performed at ICP OES, series iCAP 6000 (ThermoScientific, Cambridge, United Kingdom). Parameters of the instrument used during the measurements of the concentrations of the selected elements are provided in Table 1.

Table 1. ICP OES parameters

Parameters	Values
RF generator power (W)	1150
Peristaltic pump velocity (rpm)	50
Nebulizer gas flow (L/min)	0.5
Washing time (s)	30
Number of measurements	3

The following standards were used for the calibration of the instrument: multielement standard solution IV for ICP, TraceCERT, Fluka Analytical, Switzerland (Al, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Tl, V and Zn); multielement standard solution III for ICP, TraceCERT, Fluka Analytical, Switzerland (Ca, K, Mg, and Na); standard Si, TraceCERT, Fluka Analytical, Switzerland; standard P, TraceCERT, Fluka Analytical, Switzerland and standard Hg, TraceCERT, Fluka Analytical, Switzerland.

After calibration, samples of urea and NPK were analyzed at four different wavelengths. Concentrations were read at the wavelength with the best calibration curve also satisfying criteria of no interference from the left or the right side in the spectrum and with a high Signal/Background ratio. Carefully chosen wavelengths for each element and the characteristics of calibration curves (r, LOD, and LOQ) are provided in Table 2.

Table 2. Characteristics of the calibration curves

Element and λ (nm)	R	LOD (mg/kg)	LOQ (mg/kg)
Al 396.152	0.999907	0.0711	0.2370
As 189.042	0.999979	0.0647	0.2155
B 249.773	0.999984	0.0177	0.0591
Ba 455.403	0.999949	0.0019	0.0062
Be 313.107	0.999995	0.0013	0.0043
Ca 393.366	0.999921	0.0056	0.0186
Cd 226.502	0.999996	0.0062	0.0206
Co 228.616	0.999998	0.0106	0.0354
Cr 283.563	0.999995	0.0169	0.0564
Cr 267. 716	0.999943	0.0254	0.0847
Cu 324.754	0.999993	0.0216	0.0719
Fe 238.204	0.999993	0.0191	0.0635
Mg 279.553	0.999996	0.0116	0.0386
Mn 257.610	0.999953	0.0039	0.0130
Na 588.995	0.999982	0.0012	0.0038
Ni 231.604	0.999980	0.0133	0.0442
P 213.618	0.999975	0.0930	0.3101
Pb 220.353	0.999998	0.0587	0.1958
Si 251.611	0.999938	0.0428	0.1427
V 309.311	0.999989	0.0120	0.0399
Zn 202.548	0.999996	0.0032	0.0106

Preparation of commercial fertilizers for ICP OES analysis

Samples of NPK 16:16:16 (SPECIAL, elixir Zorka) as an inorganic mineral fertilizer and urea (EK MŰTRAGYA, 46 % N) as an organic fertilizer were ground in the mortar and dried at 120°C for 5 h. Two methods were used for the dissolution of these fertilizers to determine the efficacy of the element extractions.

Method I: Dissolution of solid commercial fertilizers with 18.5 % HCl

Approximately 1 g of urea and NPK were measured in Erlenmeyer flasks (150 ml), then added 10 ml 18.5 % HCl (Sigma-Aldrich, Germany). The content was warmed at a hot plate for around 20 min, then cooled to room temperature, filtered using blue filter paper (AHLSTROM MUNKSJÖ, Germany), and the filtrates were collected in 25 ml volumetric flasks and filled up with deionized water (ThermoScientific, 2017).

Method II: Dissolution of solid commercial fertilizers with aqua regia

Approximately 1 g of urea and NPK were measured in Erlenmeyer flasks (150 ml), then added 10 ml aqua regia (the mixture conc. HCl (Sigma-Aldrich, Germany) and conc. HNO_3 (Macron, Germany) in the ratio 3:1). The content was warmed at a hot plate and evaporated to dryness. Afterward, 5 ml conc. HCl (Sigma-Aldrich, Germany) was added to both samples and warmed around 5 min until the dissolution of the dry residues. The samples were cooled, filtered through the blue filter paper (AHLSTROM MUNKSJÖ, Germany), and the filtrates were collected in 25 ml volumetric flasks and filled up with deionized water (https://www.ssi.shimadzu.com/sites/ssi.shimadzu.com/files/Products/literature/ICP/J092.pdf).

Microsoft Office 2010 package was used to process and display the results, and the results are represented as mg of element per kilogram of dry fertilizer sample.

Results and Discussion

In this paper, it was investigated the extraction of selected elements from mineral NPK fertilizer and urea as an organic fertilizer using two different methods of the preparation of samples of solid fertilizers. Concentrations of determined elements are shown in Table 3. Based on the obtained results, high concentrations of the elements were observed in the samples of NPK fertilizers (independently on the preparation method) compared to the values of the concentrations of elements in the samples of urea. These results are thought-provoking, and they are telling us that NPK fertilizer was unequivocally a more significant pollutant than urea, particularly since it has been used for years and continually in agriculture. It seems that more or less increased content in NPK fertilizer compared to urea most probably leads from phosphate raw material used in the process of the production of this fertilizer (Zeremski-Škorić et al., 2010).

Regarding the efficacy of the extractions of primary nutrients from NPK fertilizer (phosphorus and potassium), better extraction results for potassium are achieved using the *method I*, while method II is better for determining phosphorus. The efficacy of the investigated methods for some secondary nutrients and micronutrients of NPK fertilizer (Ca, Mg, Fe, Mn, Cu, Zn, and B) is different from one element to another. The *method I* shows a higher degree of extraction to B, Ca, and Mg, while *method II* is better for the rest of the above-mentioned elements. Co and Ni are better extracted using *method II*, but the difference is negligible.

Concentrations of As, Cr, Al, and V obtained using *methods I* and *II* do not differ significantly. It means that these two methods are the same regarding the extractions of the above-mentioned elements from NPK fertilizers.

For most elements from the 3d transition series, *method II* was shown as a better method. The reactive mixture (HNO_3:HCl=1:3) of this method, besides very high oxidative character, also contains reactive chemical species, nitrosyl cation ($\ddot{N} \equiv \ddot{O}^+$) (Safarova et al., 2011). Since metals from the 3d series of the Periodic Table of Elements show high affinity towards the ligands with N-donor atom, probably nitrosyl cation from *aqua regia* contributes to the extractions of the above-mentioned metals forming with them stable complexes.

Method II is better for extracting Ba, Co, Fe, Mg, Mn, Ni, and P from the sample of urea, while for the rest of the investigated elements except Si, this method is inferior. Based on results given in Table 3, it is observed that *method II* is better than the *method I* for the extraction of transition metals from sample NPK, but for the sample of urea, it seems that the extraction capability of *aqua regia* is weakened for these metals, and it is best reflected on Cu, Cr, and Zn. It is most probably that nitric acid from *aqua regia* during heating is spent on the degradation of urea, which leads to this outcome for this type of sample.

Zeremski-Škorić et al. (2010) determined the content of toxic metals (Cd, Cr, Ni, and Pb) in NPK fertilizers and gave the range of their concentrations in NPK fertilizers (0.52-6.38 mg/kg Cd, 0.66-127.33 mg/kg Cr, 0.91-18.16 mg/kg Ni, and 0.37-6.86 mg/kg Pb). Interestingly, our average concentrations of mentioned toxic metals are in the range of the concentrations given by these researchers.

Table 3. Average concentration ± SD (mg/kg)

Sample	Al	As	B	Ba	Be	Ca	Cd
NPK (I)	910±30	2.46±0.05	102±2	1.42±0.04	0.77±0.04	910±30	4.6±0.2
NPK (II)	920±20	2.41±0.07	96±2	1.14±0.01	0.797±0.005	650±20	4.88±0.02
Urea (I)	7.1±0.1	n.d.	5.3±0.2	0.325±0.003	n.d.	26.1±0.5	n.d.
Urea (II)	5.95±0.05	n.d.	1.86±0.03	0.352±0.005	n.d.	21.8±0.7	n.d.

Sample	Co	Cr	Cu	Fe	K	Mg	Mn
NPK (I)	1.3±0.2	69±4	7.5±0.3	3300±200	119000±10000	1120±60	194±9
NPK (II)	1.65±0.01	73.1±0.7	8.7±0.2	3560±20	87000±4000	910±20	205±2
Urea (I)	0.325±0.008	0.43±0.02	0.91±0.01	39.0±0.2	350±20	3.5±0.2	0.54±0.01
Urea (II)	0.36±0.01	0.38±0.02	0.79±0.04	45.7±0.5	132±9	3.9±0.2	0.79±0.02

Sample	Na	Ni	P	Pb	Si	V	Zn
NPK (I)	68±5	14.5±0.6	41000±3000	1.66±0.08	276±5	111±3	940±80
NPK (II)	47±2	15.218±0.005	48100±300	1.52±0.06	37.5±0.7	112±2	1070±5
Urea (I)	1.62±0.07	0.515±0.005	5.5±0.4	0.90±0.07	16.92±0.06	n.d.	7.6±0.2
Urea (II)	0.85±0.03	0.64±0.01	6.33±0.01	0.68±0.01	16.7±0.4	n.d.	6.31±0.08

(I)-Method I
(II)-Method II
n.d.-not detected

There are numerous published investigations on determining the contents of heavy (and/or toxic) metals in phosphorus fertilizers. Mortvedt et al. (1981) gave the contents of the following metals: Cd (2-153), Cu (1-18), Mn (3-73), Ni (10-156), Pb (4-8), and Zn (1-1.290) mg/kg, in diammonium phosphate (DAP) fertilizers. In our work, the average values of the concentrations are in the range of concentrations for Cd, Cu, and Ni; the concentration of manganese is higher approximately three times, lead is present in three to six times less quantities, and a significantly higher concentration of zinc is observed.

Considering that the commercial fertilizers are used without any control and for a longer time for crops and plant cultures, to increase the yield and quality of the products, they can be potential pollutants of agricultural soils. Therefore, each country gives regulations about the maximum allowed quantities of some toxic metals in commercial fertilizers. Maximum allowable concentrations (MAC) for arsenic, cadmium, chromium, nickel, lead, and mercury are given in Table 4. (Official Gazette of Serbia, No. 41/09, 2009) and Regulations for the European Union (European Union, 2019)).

Table 4. MAC (mg/kg) of some toxic elements in NPK fertilizers

Toxic element	**MAC (mg/kg)**
Arsenic (As)	40^{b}
Cadmium (Cd)	75^{a} 60^{b}
Chromium (Cr)	500^{a} 2 (Cr (VI))^{b}
Nickel (Ni)	$100^{a,b}$
Lead (Pb)	100^{a} 120^{b}
Mercury (Hg)	$1^{a,b}$

a – data from Gazette of Serbia, no. 41/09)
b – Maximum allowed value determined for the European Union.

Comparing the values of toxic elements from Table 3 with MAC values, it is observed that NPK and urea do not contain any value above the MAC, although at first sight NPK fertilizer looks contaminated with heavy metals.

Concentrations of chromium and nickel in the samples of NPK fertilizers are even around 7 times lower than MAC values recommended by Serbian regulations, and cadmium is found in even lower quantities.

Conclusion

This study compared the contents of 21 elements in two different fertilizers types: urea and NPK, using two different methods (the *method I* and *method II*) for preparing the samples. Based on the obtained results, we conclude that the contents of all elements are higher in NPK fertilizer than in urea. The extraction methods efficacy of the extractions is different from one element to another, except for the 3d elements for which its efficacy *method II*. From the primary nutrients in NPK fertilizer and urea K is more abundant than P. From the selected secondary nutrients and micronutrients in NPK fertilizer, the abundance of the elements is as follows: Fe, Mg, Ca, Zn, Mn, B, and Cu, while for urea is: Fe, Ca, Zn, Mg, B, Cu, and Mn. Concentrations of potentially toxic elements (As, Cd, Cr, and Pb) are lower than the maximum allowed value by Serbian regulation.

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Conflict-of-Interest Statement

None.

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Ekstrakcija odabranih elemenata iz komercijalnih đubriva

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SAŽETAK

Korišćene su dve metode pripreme veštačkih đubriva za ICP OES analizu na dva različita tipa đubriva: urea i NPK (mineralno đubrivo koje sadrži hranljive materije N, P i K), kao organsko i mineralno đubrivo, respektivno. Cilj ovog istraživanja bio je upoređivanje dve metode za pripremu uzoraka, određivanje sadržaja elemenata, i upoređivanje dobijenih rezultata sa maksimalno dozvoljenim koncentracijama. Prva metoda pripreme sastojala se iz digestije sa 18.5 % HCl, a druga metoda iz digestije sa carskom vodom. Za ekstrakciju nekih sekundarnih nutrienata i mikronutrienata (Ca, Mg, Fe, Mn, Cu, Zn i B) iz NPK đubriva, bolja ekstrakcija postiže se prvom metodom za B, Ca i Mg, dok je druga metoda bolja za Fe, Mn, Cu, i Zn. Rezultati analize pokazali su da je digestija carskom vodom je dobra uglavnom za sve ispitivane 3d metale iz oba uzorka, s tim što je stepen ekstrakcije nešto veći za uzorak NPK đubriva. U ovom radu određene koncentracije nekih toksičnih elemenata (As, Cr, Cd, Ni i Pb) ne prekoračuju MDK vrednosti.

Ključne reči: urea, NPK đubrivo, elementi, ICP OES

Extraction d’éléments sélectionnés à partir d’engrais commerciaux

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RÉSUMÉ

Deux méthodes de préparation pour l’analyse ICP OES des engrais commerciaux ont été utilisées pour deux types d’engrais différents : l’urée et le NPK (engrais minéral contenant des nutriments N, P et K), comme engrais organiques et inorganiques, respectivement. Le but de notre recherche était la comparaison de deux méthodes pour la préparation des échantillons, la détermination du contenu des éléments et la comparaison des résultats obtenus avec les concentrations maximales autorisées. La première méthode de préparation consiste en la digestion avec du HCl à 18,5% et l’autre méthode la digestion avec de l’eau régale. En ce qui concerne l’extraction de certains nutriments secondaires et micronutriments (Ca, Mg, Fe, Mn, Cu, Zn et B) de l’engrais NPK, une meilleure extraction a été obtenue en utilisant la première méthode pour B, Ca et Mg tandis que l’autre méthode était meilleure pour Fe, Mn, Cu et Zn. Sur la base des résultats de l’analyse, la digestion à l’eau régale est bonne pour presque tous les métaux 3d étudiés des deux échantillons, à l’exception du NPK pour lequel le degré d’extraction est un peu plus élevé. Les concentrations déterminées de certains éléments toxiques (As, Cr, Cd, Ni et Pb) ne dépassent pas les concentrations maximales admissibles (MAC).

Mots-clés : urée, engrais NPK, éléments, ICP OES

Экстракция отдельных элементов из коммерческих удобрений

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АННОТАЦИЯ

Два метода подготовки для ИСП-ОЭС анализа коммерческих удобрений использовались для двух разных типов удобрений: мочевины и НПК (минерального удобрения, содержащего питательные вещества N, P и K), в качестве органических и неорганических удобрений соответственно. Целью наших исследований было сравнение двух методов подготовки проб, определение содержания элементов и сравнение полученных результатов с предельно допустимыми концентрациями. Первый метод приготовления состоит из вываривания 18,5% HCl, а другой метод — вываривания царской водкой. Что касается экстракции некоторых вторичных питательных веществ и микроэлементов (Ca, Mg, Fe, Mn, Cu, Zn и B) из удобрений NPK, лучшая экстракция была достигнута при использовании первого метода для B, Ca и Mg, в то время как другой метод был лучше для Fe, Mn, Cu и Zn. По результатам анализа хорошо перевариваются царской водкой практически все исследованные 3d-металлы из обеих проб, за исключением N, P и K, степень экстракции которых несколько выше. Определенные концентрации некоторых токсичных элементов (As, Cr, Cd, Ni и Pb) не превышают предельно допустимые концентрации (ПДК).

Ключевые слова: мочевина, NPK удобрения, элементов, ИСП-ОЭС

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Extraktion ausgewählter Elemente aus Handelsdüngern

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ABSTRACT

Zwei Präparationsmethoden für die ICP-OES-Analyse von Handelsdüngern wurden für jeweils zwei verschiedene Düngemitteltypen verwendet: Harnstoff und NPK (Mineraldünger, der die Nährstoffe N, P und K enthält), als organische bzw. anorganische Düngemittel. Das Ziel unserer Forschung war der Vergleich zweier Methoden zur Probenvorbereitung, die Bestimmung der Elementgehalte und der Vergleich der erhaltenen Ergebnisse mit den maximal zulässigen Konzentrationswerten. Die erste Präparationsmethode besteht aus dem Aufschluss mit 18,5 %iger HCl, die andere Methode aus dem Aufschluss mit Königswasser. In Bezug auf die Extraktion einiger sekundärer Nährstoffe und Mikronährstoffe (Ca, Mg, Fe, Mn, Cu, Zn und B) aus NPK-Dünger wurde mit der ersten Methode eine bessere Extraktion für B, Ca und Mg erreicht, während sich die andere Methode besser für Fe, Mn, Cu und Zn eignete. Aus den Analyseergebnissen ist festzustellen, dass der Königswasseraufschluss für fast alle untersuchten 3d-Metalle aus beiden Proben gut ist, mit Ausnahme von NPK, bei dem der Extraktionsgrad etwas höher liegt. Die ermittelten Konzentrationswerte einiger toxischer Elemente (As, Cr, Cd, Ni und Pb) überschreiten jedoch nicht die maximal zulässigen Konzentrationswerte (MAC).

Schlüsselwörter: Harnstoff, NPK-Dünger, Elemente, ICP OES

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Determination of the invertase activity in honey samples as the indicator of the authenticity of honey by UV/VIS spectrophotometric method

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ABSTRACT

Honey is a food consumed by humans of different ages, medical conditions, and professional orientations due to its high health and nutritional properties. Due to other purposes, honey sometimes must be treated, and its values should not be lost. Adulteration of honey is frequently found on the market nowadays, so accurate methods must be used to search for honey-like products. Invertase activity is a good indicator of freshness, thermal treatment, and honey authenticity. A fast UV/VIS spectrophotometric method was modified for the implementation in the food industry for rapid determination of invertase activity of honey samples.

Keywords: invertase, honey, authenticity, etalon.

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Introduction

Honey is a complex mixture produced by honeybees (*Apis mellifera*) from floral nectar containing sugars and water and small quantities of vitamins, minerals, fatty acids, amino acids, and proteins (Hungerford et al., 2020). The purity, floral and geographical origin and authenticity are important factors regarding the quality and the price (Voica et al., 2020). Due to the reason that bees' products have an important place in the chain "toxicant-soil-plant-bee-bee product-man", honey can be used as a not expensive method for monitoring environmental pollution (Nikolov et al., 2019). Regarding the presence of natural radionuclides ^{40}K and ^{137}Cs, the investigation performed on honeys from Turkish cities shows that found activities of ^{137}Cs do not exceed the highest permitted level of the radionuclide for food slightly contributed to the natural radioactivity of honey (Altekin et al., 2015). Honey, one of the oldest sweetening agents known for wound treatment, was evaluated for anti-bacterial activities; it was found that it possesses good activity against Gram-positive pathogens (Bhushanam and Madhusudan, 2019).

The most widespread enzymes in honey are diastase (amylase), invertase, glucose oxidase, catalase, and phosphatase (Pascual-Mate et al., 2018). Invertase is produced in the hypopharyngeal gland of the honeybee (Crane, 1975). The role of invertase is to make a very concentrated solution of sugars resisting fermentation, thus representing a high-energy foodstuff occupying the minimum area in honeycombs (Crane, 1975; 1990). Invertase activity is an important parameter for estimating honey quality and freshness (Julika et al., 2020). It is a better freshness indicator than diastase number because of its easy determination, susceptibility towards heating and storage, and presence in higher quantities than diastase (Bogdanov et al., 1999; Sanchez et al., 2001; White et al., 1964).

The study aimed to evaluate the authenticity of several honeys supplied by individual honey producers to Timomed d.o.o. Knjazevac based on the invertase activity determined by the UV/VIS spectrophotometric method.

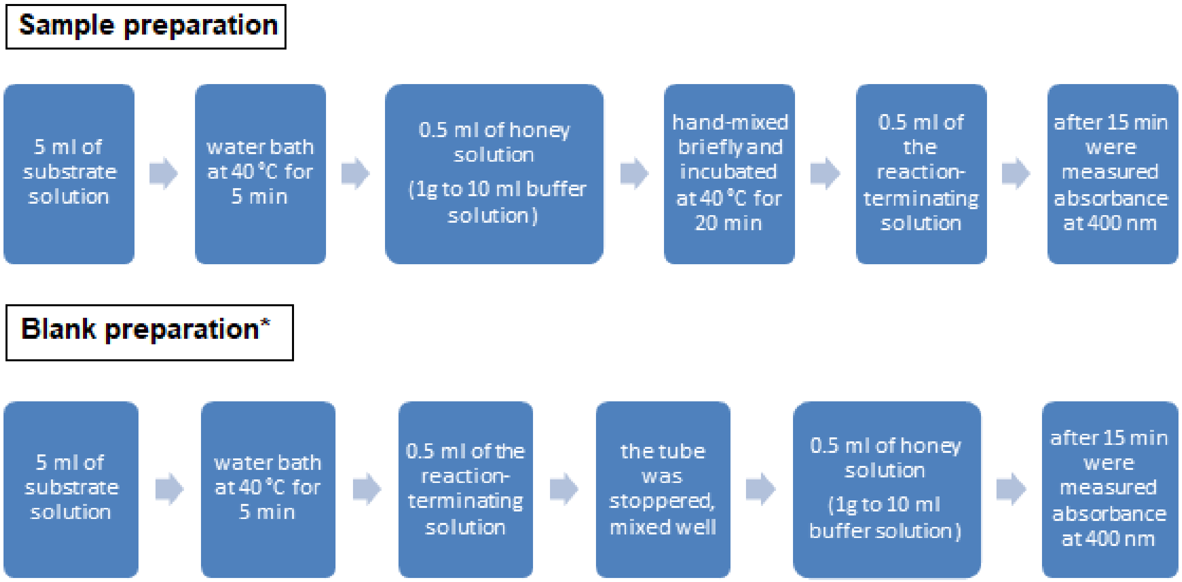
Experimental

The harmonized method for the determination of the invertase activity of the International Honey Commission (International Honey Commission, 2009) and the method approved in

Moscow 2017 (Interstate Council for Standardization, Metrology and Certification, 2017) was modified with the aim to decrease the quantity of used chemicals necessary for the determination of the invertase activity in industrial conditions.

To prepare buffer solution (0.1 M; pH = 6.0), it was dissolved 11.66 g of potassium hydrogen phosphate (KH_2PO_4) and 2.56 g of disodium hydrogen phosphate (Na_2HPO_4 x $2H_2O$) in water and diluted to 1 L. The substrate was a *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) solution. It was dissolved 1.2 g of *p*NPG in buffer solution and made up to 200 mL. *p*NPG is sparingly water-soluble, but the solution is not very stable. The buffer solution was dissolved by heating but not above 60 ^{o}C and cooled immediately after the solution was complete. Reaction-terminating solution (pH = 9.5) was made by dissolving 36.342 g of tris-(hydroxymethyl) aminomethane in water and diluting to 100 mL. A pH-value of 9.5 was adjusted with conc. hydrochloric acid.

Sample preparation and blank preparation are nicely described in Figure 1.



*A separate blank for each honey tested was prepared (Bogdanov et al., 1997).

Figure 1. Schematic representation of the sample and blank preparation process

The absorbance of the blank was subtracted from that of the sample solution (ΔA400). The amount of *p*-nitrophenol in µM produced during the test corresponds exactly to the amount of substrate in µM utilized. Therefore, the honey invertase activity (IA) can be calculated from the absorbance measured at 400 nm and is indicated in units/kg (U/kg):

$$\text{IA (U/kg)} = 2 \times (6 \times 0.05 \times 0.05298 \times 10^{4} \times \Delta A) = 317.88 \times \Delta A$$

where 6 = factor for the ml of sample solution used (total volume), 0.05 = converts reaction time from 20 min to 1 min, 10^4 = converts the amount of honey taken to 1 kg, 0.05298 = conversion factor for µg into µM per ml, 2 = conversion factor for honey quantity.

This method (Figure 1) was later again modified, and the modifications include 2.5 ml *p*NPG solution, 0.25 ml honey solution and 0.25 ml reaction-terminating solution. The invertase activity, in that case, was calculated according to:

$$\mathrm{IA\ (U/kg)} = 4 \times (3 \times 0.05 \times 0.05298 \times 10^4 \times \Delta A) = 317.88 \times \Delta A$$

where 3 = factor for the ml of sample solution used (total volume), 0.05 = converts reaction time from 20 min to 1 min, 10^4 = converts the amount of honey taken to 1 kg, 0.05298 = conversion factor for µg into µM per ml, 4 = conversion factor for honey quantity.

It is common to express the invertase activity as invertase number (IN). The IN indicates the amount of sucrose per g hydrolyzed in 1 hour by the enzymes contained in 100 g of honey under test conditions (Hadorn and Zürcher, 1966). The relation between U/kg and IN (Oddo et al., 1999) is:

$$\mathrm{IA\ (U/kg)} = 7.345 \times \mathrm{IA}' \mathrm{\ (IN)}$$

Results and Discussion

Nine samples from individual honey producers supplied to Timomed d.o.o. Knjazevac were analyzed in the laboratory at the Department of Chemistry, Faculty of Sciences and Mathematics, University of Nis, Nis (Table 1), where the method for the determination of the invertase activity in honey samples was developed. Except for one sample with the found invertase activity negative (-6.83 U/kg, -0.93 IN), all other eight samples show positive values going from 30.36-167.68 U/kg (4.13-22.83 IN).

Table 1. Invertase activity of selected honey samples analyzed at Department of Chemistry, Faculty of Sciences and Mathematics, University of Nis

Sample	Total solution volume (ml)	A_{blanc} (average) ±SD*	A_{sample} (average)±SD**	ΔA	Invertase activity (U/kg)	Invertase activity (IN)
1	6	0.482±0.002	1.01±0.05	0.5275	167.68	22.83
2	6	0.41±0.06	0.506±0.009	0.0955	30.36	4.13
3	6	0.477±0.001	0.637±0.006	0.1600	50.86	6.92
4	6	0.43±0.05	0.41±0.04	-0.0215	-6.83	-0.93
5	6	0.392±0.007	0.55±0.06	0.1620	51.50	7.01
6	3	0.6±0.1	0.97±0.06	0.4195	133.35	18.16
7	3	0.54±0.07	0.68±0.08	0.1440	45.77	6.23
8	3	0.4875±0.0007	0.6±0.1	0.1645	52.29	7.12
9	3	0.59±0.03	0.94±0.09	0.3565	113.32	15.43

*n=2, n-number of measurements

**n=3, n-number of measurements

SD-standard deviation

The invertase activity of another five different honey samples supplied from individual honey producers to Timomed d.o.o. Knjazevac was determined in the laboratory at Timomed d.o.o. Knjazevac using the developed method and shows values from 45.62-245.72 U/kg. The invertase number goes from 6.21 to 33.45 IN (Table 2). Due to the reason that industry needs not such precise values to decline/accept the honey from the supplier, one blank probe was used and two probes of the sample.

Table 2. Invertase activity of selected honey samples analyzed at Timomed d.o.o. Knjazevac

Sample	**Total solution volume (ml)**	**A_{blanc}**	**A_{sample} (average)±SD***	**ΔA**	**Invertase activity (U/kg)**	**Invertase activity (IN)**
10	3	0.449	0.592±0.008	0.1435	45.62	6.21
11	3	0.482	1.26±0.02	0.7730	245.72	33.45
12	3	0.450	0.69±0.02	0.2355	74.86	10.19
13	3	0.444	0.7±0.1	0.2650	84.24	11.47
14	3	0.448	0.6360±0.0000	0.1880	59.76	8.14

[*] n=2, n-number of measurements

SD-standard deviation

Eleven analyzed samples have invertase activity of more than 4 and less than 20 IN, which is following the research performed by Oddo et al. (1999). One sample with a colour different from others (darker) has an invertase activity of more than 30, which is a characteristic of honeydew honey (Oddo et al., 1999). Honeydew honey and honeydew, which honey bees collect from trees and leaves of conifers and deciduous trees, are a subject of numerous studies (Markwell et al., 1993; Martins-Mansani et al., 2021; Pita-Calvo and Vazquez, 2018; Santas, 1983; Shaaban, 2020; Ülgentürk et al., 2020; Ünal et al., 2017; Utzeri et al., 2018). The higher invertase activity of honeydew honey compared to other honey types can be explained by the high presence of enzymes (particularly invertase) in raw material (honeydew) which has the origin in the secretions of salivary glands and the gut of plant-sucking insects (Oddo et al., 1999). Unfortunately, one analyzed sample showed bizarre behaviour, and that sample is not honey in any sense (-0.93 IN).

The variability in invertase activity can be a result of a series of factors (*e.g.*, nectar collection period, an abundance of nectar flow and its sugar content, age of the bees, pollen consumption, *etc.* (Brouwers, 1982; 1983; Fluri et al., 1982; Huang et al., 1989a, b; Oddo et al., 1999; Simpson et al., 1968).

Nowadays, the adulteration of honey is a common feature, and their producers using the various methods tend to destroy the excess of the enzyme invertase. It was shown that long processing times and high temperatures (> 6h and > 65 ^{0}C) were necessary to start the inactivation of the enzyme invertase (Sahin et al., 2020). Therefore, it is logical that adulterated honey would have lower IN than natural. We can suspect that samples 2, 3, 5, 7, 8, 10 and 14 are not entirely natural based on the recommendation of the International Honey Commission (IN>10). However, they can be honeys with low enzymatic activity (IN>4) (Bogdanov et al., 1999). On the other hand, various processing operations have been introduced over the last decades to ensure a pleasant and homogeneous presentation of the product in liquid or semisolid form requiring heating (Serra Bonvehı et al., 2000; Vicente Pascual et al., 1987). Various heating methods were proposed decreasing to a small extent the invertase activity but changing the product in the desired direction (Serra Bonvehı et al., 2000).

The absorbance of blank samples for selected honey samples varies from 0.4 to 0.6 (Tables 1 and 2). We suggest the use of blank solutions of true bee honeys (those have IN very close to 4 and 20) as etalons for the comparison with the blank solution of the unknown sample with the satisfactory electrical conductivity (due to the existence of the correlation of minerals (contributing significantly to the electrical conductivity of honeys) and invertase) (Vorlova and Celechovska, 2002) and taste without the use of the equipment, such as UV/VIS, using only visual inspection due to coloration. The color of the blank is not enough, as it was seen in the case of sample 4 where the color was artificial, and no invertase was present there. Therefore, for the rapid determination, some other additional fast measurement must be used as suggested to be complementary method to determine the authenticity of honey sample if we do not have time for a full UV-VIS spectrophotometric determination including sample and blank preparation as described in this work.

Conclusion

Invertase activity has been shown to be a good indicator of honey's freshness, thermal treatment, and authenticity in the food industry. The developed UV/VIS spectrophotometric method is fast and easy to use in academia and particularly in industry. The absorbance of the blank sample can tell a lot about the quality of the honey (together with electrical conductivity and the taste) and sometimes, in a hurry, the blank solutions of the authentic honeys can be etalons for the comparison with a blank solution of the unknown sample (with proper electrical conductivity and the taste) thus without the use of equipment such as UV/VIS.

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Conflict-of-Interest Statement

None.

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Određivanje aktivnosti invertaze u uzorcima meda kao indikatora autentičnosti meda korišćenjem UV/VIS spektrofotometrijske metode

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SAŽETAK

Med je namirnica koju zbog njenih zdravstvenih i nutritivnih svojstava upotrebljavaju ljudi različite starosti, zdravstvenog stanja i profesije. Za drugačije namene med se ponekad mora tretirati, a njegove vrednosti pri tome ne bi trebalo izgubiti. Krivotvorenje meda danas je često na tržištu, pa treba koristiti precizne metode za otkrivanje proizvoda nalik medu. Aktivnost invertaze je dobar indikator za svežinu, termički tretman i autentičnost meda. Brza UV/VIS spektrofotometrijska metoda razvijena je sa ciljem da bude primenjena u industriji hrane radi brzog određivanja aktivnosti invertaze u uzorcima meda.

Ključne reči: invertaza, med, autentičnost, etalon

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Détermination de l’activité invertase dans des échantillons de miel comme indicateur de l’authenticité du miel par méthode spectrophotométrique UV/VIS

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RÉSUMÉ

Le miel est un aliment consommé par des gens d’âge différent, de conditions médicales et d’orientations professionnelles différentes en raison de ses propriétés nutritionnelles et sanitaires élevées. À d’autres fins, le miel doit parfois être traité et ses valeurs ne doivent pas être perdues. L’adultération du miel se trouve fréquemment sur le marché de nos jours, des méthodes précises doivent donc être utilisées pour rechercher des produits similaires au miel. L’activité invertase est un bon indicateur de fraîcheur, de traitement thermique et d’authenticité du miel. Une méthode spectrophotométrique UV/VIS rapide a été développée pour être mise en œuvre dans l’industrie alimentaire pour la détermination rapide de l’activité invertase des échantillons de miel.

Mots-clés : invertase, miel, authenticité, étalon

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Определение активности инвертазы в образцах меда как индикатора подлинности меда спектрофотометрическим методом UV/VIS

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АННОТАЦИЯ

Мед является продуктом питания, потребляемым людьми разного возраста, состояния, здоровья и профессиональной ориентации в связи с его высокими оздоровительными и питательными свойствами. Для других целей мед иногда необходимо обрабатывать, и его ценность не должна теряться. В настоящее время на рынке часто встречается подделка меда, поэтому для поиска медоподобных продуктов необходимо использовать точные методы. Активность инвертазы является хорошим показателем свежести, термической обработки и подлинности меда. Быстрый спектрофотометрический метод UV/VIS был разработан для применения в пищевой промышленности для определения активности инвертазы в образцах меда.

Ключевые слова: инвертаза, мед, подлинность, эталон

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Bestimmung der Invertase-Aktivität in Honigproben als Indikator für die Authentizität von Honig mittels UV/VIS-Spektrophotometrie

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ABSTRACT

Honig ist ein Lebensmittel, welches aufgrund seiner hohen Gesundheits- und Nährwerteigenschaften von Menschen unterschiedlichen Alters, Gesundheitszustands und Berufsorientierungen konsumiert wird. Wegen anderer Verwendungszwecke muss Honig manchmal behandelt werden, und seine Werte sollten dabei nicht verloren gehen. Honigverfälschungen sind heutzutage häufig auf dem Markt aufzufinden, so dass genaue Methoden für die Suche nach honigähnlichen Produkten angewandt werden müssen. Die Invertase-Aktivität ist ein guter Indikator für Frische, thermische Behandlung und Honigauthentizität. Es wurde eine schnelle UV/VIS-spektrophotometrische Methode entwickelt, die in der Lebensmittelindustrie zur schnellen Bestimmung der Invertase-Aktivität von Honigproben eingesetzt werden kann.

Schlüsselwörter: Invertase, Honig, Authentizität, Etalon

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