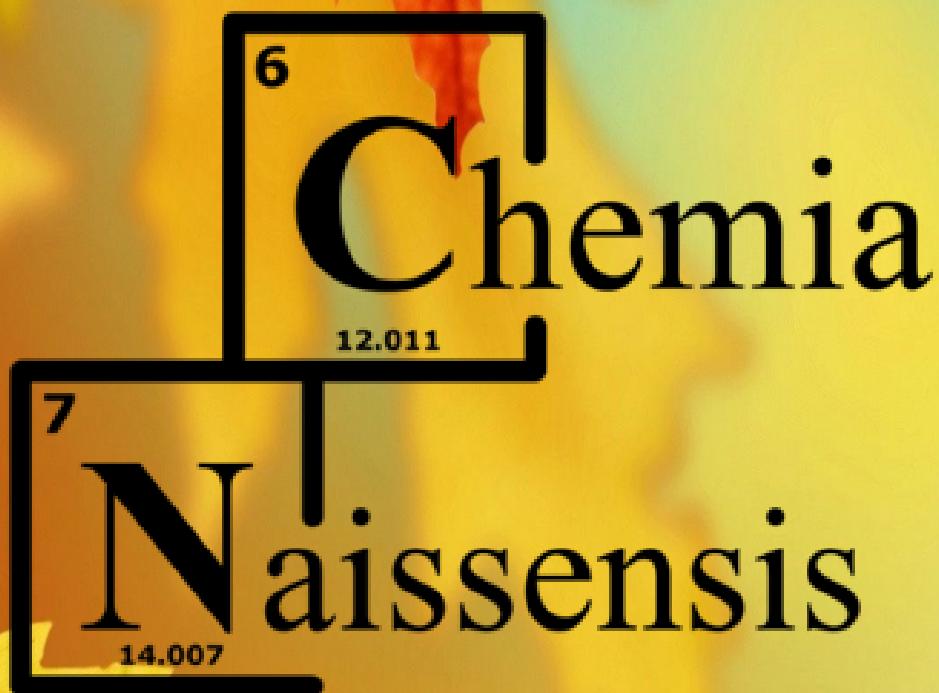


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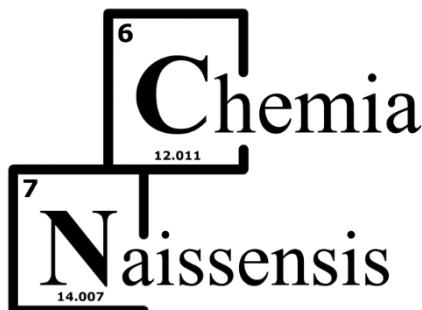
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Calculating stabilization energies for aromatic molecules in schools - quantum chemistry, a valuable tool for teaching and education

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ABSTRACT

While aromaticity is one of the most fundamental concepts in chemistry and is taught in many elementary organic chemistry courses, it is hardly possible to activate pupils and students to learn and explore this topic independently. By applying computational chemistry and especially quantum chemistry, we introduce a possibility to activate students, allowing them to test taught

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concepts and do their own “experiments” cheaply and securely. We have focused in the current report on the structural and energetic aspects of aromaticity, as they are most compatible with other topics of typical chemistry lessons. To evaluate the aromatic stabilization energy, we suggest the simple-to-calculate and easy-to-understand isomerization stabilization energy (ISE) popularized by Paul von Ragué Schleyer and Frank Pühlhofer some 20 years ago. As practical examples, we demonstrate our concept with benzene- and pyridine-based systems and suggest topics for small projects for students and pupils.

Keywords: *aromaticity, computational chemistry experiments, education*

Introduction

Aromaticity is one of the fundamental concepts in chemistry, initially explaining the special behavior of benzene (Badger, 1969; Garratt, 1971), nowadays ubiquitous in all chemical subdisciplines (Boldyrev et al., 2005; Chattaraj, 2011; Mercero et al., 2015). In schools, the topic “aromaticity” is taught based on Hückel’s theory (Hückel, 1931a; Hückel, 1931b; Hückel, 1932) and the $4n+2 \pi$ -rule clearly and simply (McMurry, 2021), leading pupils to the idea that they and all others have understood aromaticity completely. More than 12,000 publications with the keyword “aromaticity” registered in the Web of Science for the last 50 years draw a different picture.

While aromaticity is in school and elsewhere in chemistry-related science, an important concept utilized, *e.g.*, in Biology to understand the NAD⁺/NADH-system (Schaller, 2021), the possibilities to enrich the lectures by attractive experiments are very limited and therefore more or less only done in theory by paper and pen or chalk and blackboard. Depending on the scientific level of pupils or students, orbitals are included, or otherwise, the explanation is limited to the theory of single and double bonds. Normally the students get a list of typical characteristics for aromatic systems and the second list of properties related to the aromatic character (McMurry, 2021) (*vide infra: Results and Discussion*), and then the identification of aromatic molecules is typically exercised by homework. Motivating lectures are something different.

A possible alternative way is to illustrate the teaching of this topic, also potentially activating pupils to own work and “independent research” by applying computational, especially quantum chemical, calculations (Simpson et. al., 2020). These methods have many benefits for teaching, i) they utilize equipment handy in many classrooms nowadays, the personal computer, and therefore do not cause extra costs ii) the necessary software is also free of charge and legally accessible and iii) no potentially dangerous chemicals are necessary. In the last years, a lot of money was spent worldwide to equip schools with contemporary personal computers for all kinds of purposes and with computer power allowing quantum chemical calculations. Additional appropriate free software is accessible free of charge, *e.g.*, there are a couple of free quantum

chemical programs like Gamess (Gordon et. al., 2005; Schmidt et al., 1993), Orca (Neese, 2012; Neese, 2017), Dalton (Aidas et al., 2014) or OpenMopac (Stewart, 2016) and also free software for generating inputs and visualizing the outputs, *e.g.*, Avogadro (Hanwell et al., 2012) can easily be obtained.

In this manuscript, some possibilities offered by the application of quantum chemical calculations to enrich the topic of aromaticity in chemistry courses shall be introduced.

Applied Method and Software

The examples introduced here were calculated with Gaussian16 (Frisch et al., 2016), and whenever possible, in G3 (Curtiss et al., 1998) and/or MP2/def2QZVPP (Hehre et al., 1986; Weigand et al., 2005; Weigand, 2006) theory, to get good reference energies. Of course, the standard density functionals like B3LYP (Becke et al., 1993; Lee et al., 1988; Stephens et al., 1994) or ωB97XD (Chai & Head-Gordon, 2008) in combination with standard basis sets like 6-311+G** or 6-31G* (Hehre et al., 1986) lead to sufficient results, too. Semiempiric methods like AM1 (Dewar et al., 1985) are also leading to correct qualitative results and can be done directly in the lecture. The results presented here were calculated with Caddle/Empire (Hennemann et. al., 2020). And beyond quantum chemistry, even MMFF94 (Thomas & Halgren, 1996) force field calculations, as implemented in Avogadro, can illustrate a lecture.

Results and Discussion

In school, aromaticity is explained with the well-known Hückel theory (McMurry, 2021). This is understandable and applicable for pupils, as it allows paper and pen to identify aromatic molecules or aromatic fragments of molecules. As properties originated by the aromatic character and therefore an indication for the aromaticity of a molecule the following four (+ one) topics (Schleyer et al., 1996) are listed below, which, in most cases, expressed without giving pupils or students the chance to verify only one of them easily by themselves.

- a) Criterion “Structure”: Deals with bond length equalization due to cyclic delocalization.
- b) Criterion “Energy”: Deals with enhanced stability (large resonance energy).

- c) Criterion “Magnetic properties”: Tests the "ring current"-effects
- d) Criterion “Chemical behaviour”: Studies the electrophilic aromatic substitution.
- e) Criterion “Smell”

Of course, for security and hazardous reasons, the smell of a compound is no more an applicable criterion to distinguish between an aromatic compound and a non-aromatic one; this criterion nowadays has only historical importance. The flavourful smell of compounds like benzene motivated August Wilhelm Hofmann in 1855 to call such compounds “aromatic” and the phenomena “Aromaticity” (Hofmann, 1855). Even if we know, nowadays there is no general relationship between aromaticity and the olfactory properties of a molecule, a hint to the smell and structure of vanillin (4-hydroxy-3-methoxybenzaldehyde) is still instructive.

Testing chemical reactivity requires lab and laboratory skills. If a lab course is included in the curriculum, it is a good idea to demonstrate and teach it experimentally, too. This is neither possible nor included in the official curriculum in many secondary schools. Additionally, many aromatic molecules do not undergo the typical textbook electrophilic aromatic substitution reactions (McMurry, 2021), *e.g.*, C₆₀. What shall be substituted? One can add bromine to phenanthrene and anthracene to point out two of the most striking examples (Price, 1936).

Of the remaining three criteria, the magnetic criterium is indeed the most difficult and abstract one to teach. The concept of John People et al. is very supportive here to understand the resonance of the alternating single and double bonds, *e.g.*, in benzene as a ring current (People and Untch, 1966). This idea of a ring current can lead pupils with a solid technical and physical background to understand aromatic molecules as a solenoid generating a magnetic field (McMurry, 2021). Surely it needs outstanding pupils to go deeper into this topic, but it is questionable if this is necessary for a secondary school.

Criteria of (a) structure and (b) resonance energy are suited to be taught and exercised by applying quantum chemical calculations during the course or in an extra exercise.

Aromaticity is connected to bond length equalization due to cyclic delocalization. While we draw benzene classically with alternating double and single bonds to explain later to students

that there are no shorter (double) and longer (single) bonds and that we have averaged bond length between the carbon atoms (Figure 1).

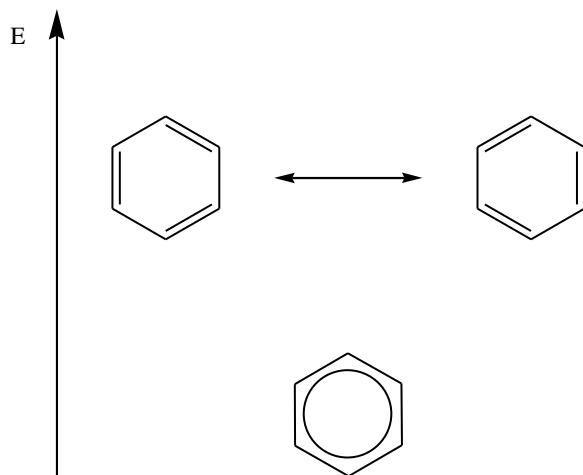


Figure 1. Resonance structures of benzene leading to the structure with bond equalization

This effect can easily be demonstrated or better evaluated by the pupils themselves. No matter how pronounced they make double and single bonds in their input file, they will end up with the well-known high symmetric D_{6h} -structure (within the accuracy of the selected method, normally two significant figures). Additionally, by comparing with simple references like cyclohexane or ethene, they can understand that the resulting bonds are neither single nor double bonds but in-between.

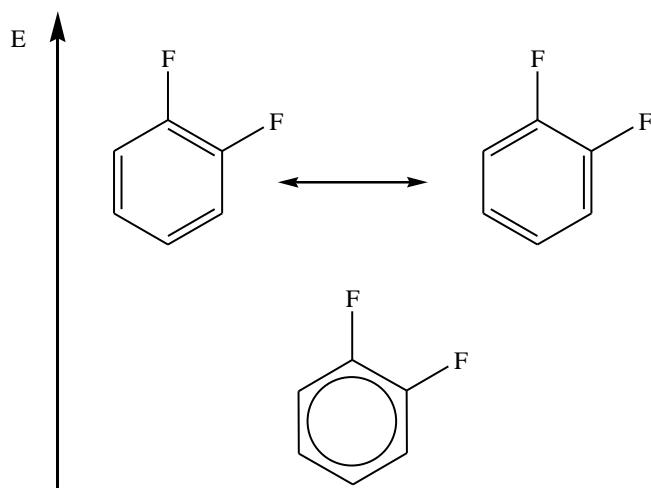
Table 1. Calculated C-C-bond length in benzene

Method	C-C bond length in benzene	C-C bond length in cyclohexane ^{cc}	C-C bond length in ethene
MMF94	1.395 Å	1.526 Å	1.336 Å
AM1	1.395 Å	1.515 Å	1.326 Å
HF/STO-3G	1.387 Å	1.544 Å	1.306 Å
HF/3-21G	1.385 Å	1.541 Å	1.315 Å
B3LYP/6-31G*	1.397 Å	1.537 Å	1.331 Å
B3LYP/6-311+G**	1.395 Å	1.536 Å	1.329 Å

$\text{@B97XD/6-311+G}^{**}$	1.390 Å	1.530 Å	1.326 Å
MP2(full)/def2QZVPP	1.387 Å	1.518 Å	1.326 Å

cc: chair conformation

As simply observable, we get with all methods an averaged C-C-bond length in benzene of 1.4 Å independent from the applied technique and, therefore, clearly significantly shorter than 1.5 Å in cyclohexane or longer than 1.3 Å in ethene.

**Figure 2.** Resonance structures of 1,2-difluorobenzene

Of course, if the resonance structure with alternating single and double bonds would be real structures, one must get two distinguished isomers, *e.g.*, in 1,2-difluorobenzene, one with a single bond between the CF-groups and one with a double bond between the CF-groups. Obviously, only one structure can be found no matter how one tries (Figure 2).

Table 2. Calculated C_F-C_F -bond length in 1,2-difluorobenzene

Method	C_F-C_F bond length in benzene (Å)
MMF94	1.397
AM1	1.421
HF/STO-3G	1.407
HF/3-21G	1.375
B3LYP/6-31G*	1.395

B3LYP/6-311+G**	1.391
ωB97XD/6-311+G**	1.388
MP2(full)/def2QZVPP	1.384

A more detailed inspection of the 1,2-difluorobenzene structures shows that the C-C-bonds are not identical anymore, obviously due to the two substituents. Comparing the non-identical bond and the structure, students will still understand that the prerequisite of Hückel's theory is still valid and that a molecule like 1,2-difluorobenzene is still aromatic.

Extending the idea that the structure can undergo perturbation by substituents can easily lead pupils and students to hetero aromatic systems like pyridine (Figure 3). A valuable tool is the application of the concept of isoelectric groups, *e.g.*, N instead CH.

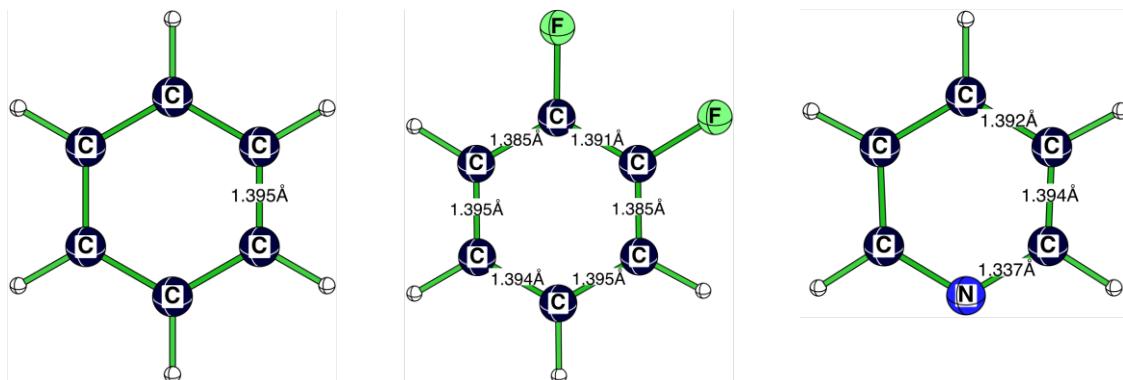
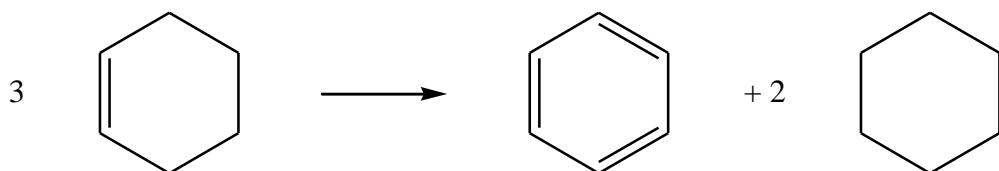


Figure 3. Calculated (B3LYP/6-311+G**) structures of benzene, 1,2-difluorobenzene and pyridine

If computer power and exercise time are available, it can be a nice challenge to ask pupils to design and calculate an aromatic molecule with a maximum bond length alternation.

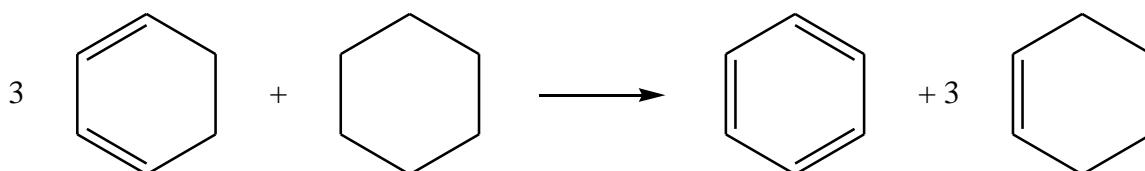
Calculating the resonance energy includes more thinking about science than playing with science, but usually students and pupils are motivated to investigate a topic new to them independently. Traditionally the aromatic stabilization energy (ASE) is calculated by model equations.

In the middle of the 20th century, Wheland suggested calculating the resonance energy by equation 1 (Wheland, 1955).



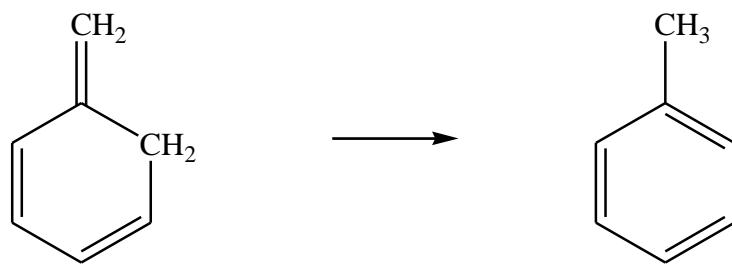
Equation 1

This equation allows measuring the total stabilization referencing to a hypothetical system without resonance. The weakness of this equation is it does not include influences like strain, hyperconjugation, differences in the types of bonds, *etc.* (Dewar and Schmeising, 1959). Stepwise new equations were suggested and applied to include all necessary effects, leading to the homodesmotic equation 2 (Schleyer and Pühlhofer, 2002).



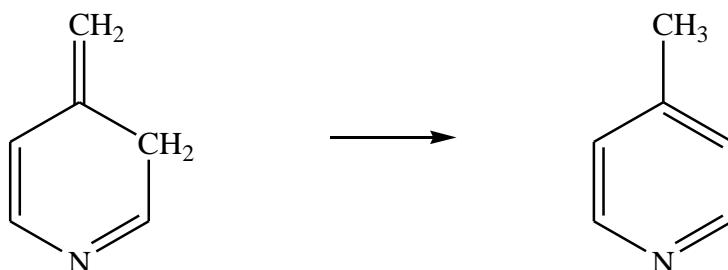
Equation 2

While accurate energy values can be calculated by all kinds of computational methods, the idea of this equation can be applied to all kinds of aromatic molecules. The significant disadvantage of a homodesmotic equation like 2 is that for the simple molecule benzene, one needs three different molecules besides benzene; in the case of pyridine, even six reference structures are necessary beside pyridine. This is for scientists unpleasant and for educational purposes prohibitive. The simple and nearly self-explanatory solution was introduced by Paul von Ragué Schleyer and Frank Pühlhofer in 2002, inspired by earlier work (Schleyer & Pühlhofer, 2002). They isomerized 5-methylenecyclohexa-1,3-diene by a hydrogen shift to methylbenzene (toluene) (equation 3) and calculated the isomerization stabilization energy (ISE). The energy difference of both isomers is the stabilization caused by cyclic electron delocalization (Schleyer & Pühlhofer, 2002).



Equation 3

Following Schleyer and Pühlhofer's approach, even for cases like pyridine, simple and fast equations are accessible, allowing students to recalculate the resonance energy themselves.



Equation 4

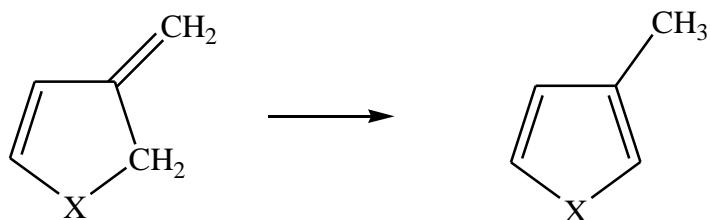
Table 3. Calculated isomerization stabilization energies (ISE) to derive the aromatic stabilization energy for benzene and pyridine

Method	ISE benzene (equation 3) (kcal/mol)	ISE pyridine (equation 4) (kcal/mol)
AM1	-24.25	-22.11
HF/STO-3G	-40.03	-38.35
HF/3-21G	-35.97	-34.99
B3LYP/6-31G*	-34.64	-33.87
B3LYP/6-311+G**	-33.24	-32.41
oB97XD/6-311+G**	-33.77	-32.97
MP2(full)/def2QZVPP	-37.49	-36.29
G3	-32.00	-31.29

A potential problem can be the position of the CH₂ group in the reference molecule relative to the methylene moiety. As demonstrated by Schleyer and Pühlhofer, impressively are, all necessary effects included in the ISE as long as the two relevant CH₂ moieties are adjacent.

At this point, students and pupils can start with small independent projects.

- a) Investigating the ISE of larger annulenes starting from benzene, *via* [10] annulene, [14] annulene, *etc.* The results one can expect are published by Schleyer et al. (2003).
- b) Calculating the ISE for smaller heteroaromatic systems ring.

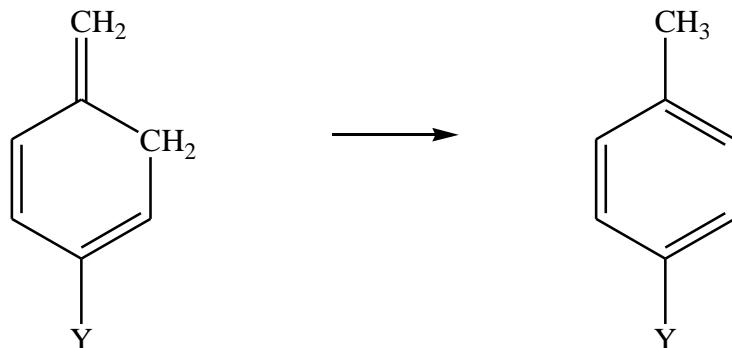


Equation 5

Table 4. G3 ISE values for heteroaromatic ring systems according to equation 5

X	BH	⁺ CH	⁻ CH	NH	PH	O	S	Se
ISE kcal/mol	+11.14	+21.37	-27.01	-21.85	-6.07	-15.50	-18.55	-14.57

- c) Studying the influences of substituents on the ISE.



Equation 6

Table 5. G3 ISE values for differently substituted benzene rings according to equation 6

Y	CH ₃	F	Cl	Br	OH	SH	SeH	NH ₂
ISE kcal/mol	-31,29	-31,90	-31,82	-31,79	-31,38	-31,09	-31,20	-31,86

Conclusion

Based on two typical criteria for aromaticity, structure (bond length equalization), and enhanced stability, we designed some simple and fast experiments for pupils and students based on computational chemistry, especially quantum chemistry. Without any hazards, the students can calculate structures and evaluate energies. While classical concepts in deriving aromatic stabilization, energies tend to be complex and time-consuming, we recommend for educational use the simple and nearly self-explanatory isomerization stabilization energy (ISE), a simple concept established for common use by Paul von Ragué Schleyer and Frank Pühlhofer. Only an H-atom is shifted from a methyl group to a carbon atom in the aromatic ring, and consequently, the ring current is stopped by the CH₂- and the exocyclic =CH₂ group. Small projects based on the structural and energetic criteria for aromaticity are suggested for the learners.

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Dedicated to StD Heinz Nikolaus Bayerlein on the occasion of his 70th birthday.

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Izračunavanje stabilizacionih energija za aromatične molekule u školama – Kvantna hemija, značajno oruđe za predavanje i obrazovanje

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SAŽETAK

Aromatičnost spada u jedan od fundamentalnih koncepta u razumevanju hemije. Iako prisutna u mnogim elementarnim organskim hemijskim kursevima, veoma je teško podstaći učenike i studente da nezavisno van svojih predavanja i dalje istražuju temu aromatičnosti jedinjenja. Primenom kompjuterske hemije, a naročito kvantne kompjuterske hemije, u ovom radu predstavljamo mogućnost da se studenti više zainteresuju za polje aromatičnosti u hemiji, omogućavajući im da individualno testiraju naučne koncepte i rade sopstvene “eksperimente” na jednostavan i siguran način. U okviru ovog rada fokusirali smo se samo na strukturni i energetski

aspekt aromatičnosti jedinjenja, jer su ovi aspekti najkompatibilniji sa ostalim lekcijama tipičnog časa hemije. Da bi odredili energiju stabilizacije uzrokovane od strane aromatičnosti sistema, predstavljamo izomerizacionu energiju stabilizacije (engl. isomerisation stabilization energy (ISE)), koja je po sebi jednostavna za računanje kao i razumevanje, a popularizovana je od strane Paula fon Rage Šlajera (Paul von Ragué Schleyer) i Franka Pilhofera (Frank Pühlhofer) pre 20 godina. Kao praktične primere predstavljamo naš koncept benzenskih i piridinskih sistema, i predlažemo teme za male istraživačke projekte studentima i đacima.

Ključne reči: aromatičnost, eksperimenti kompjuterske hemije, obrazovanje

Calcul des énergies de stabilisation des molécules aromatiques dans les écoles - la chimie quantique, un outil précieux pour l'enseignement et l'éducation

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RÉSUMÉ

Alors que l'aromaticité est l'un des concepts les plus fondamentaux de la chimie et elle est enseignée dans de nombreux cours élémentaires de chimie organique, il n'est guère possible d'inciter les élèves et les étudiants à apprendre et à explorer ce sujet de manière indépendante. En

appliquant la chimie computationnelle et en particulier la chimie quantique, nous introduisons une possibilité d'activer les étudiants en leur permettant de tester les concepts enseignés et de faire leurs propres "expériences" à moindre coût et en toute sécurité. Nous nous sommes concentrés dans le rapport actuel sur les aspects structurels et énergétiques de l'aromaticité, car ils sont les plus compatibles avec d'autres sujets de cours de chimie typiques. Pour évaluer l'énergie de stabilisation aromatique, nous suggérons l'énergie de stabilisation d'isomérisation (ISE) simple à calculer et facile à comprendre, popularisée par Paul von Ragué Schleyer et Frank Pühlhofer il y a une vingtaine d'années. Comme exemples pratiques, nous démontrons notre concept avec des systèmes à base de benzène et de pyridine et suggérons des sujets pour de petits projets pour les étudiants et les élèves.

Mots-clés: aromaticité, expériences de chimie computationnelle, éducation

Расчет энергий стабилизации ароматических молекул в школах - квантовая химия, ценный инструмент для обучения и образования

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АННОТАЦИЯ

Ароматичность относится к одному из фундаментальных понятий в понимании химии, хотя она и присутствует во многих курсах элементарной органической химии, очень трудно побудить школьников и студентов к самостоятельному изучению темы ароматичности соединений вне лекций. Применяя вычислительную химию и особенно квантовую химию, мы представляем возможность активизировать студентов, позволяя им проверять преподаваемые концепции и проводить свои собственные «эксперименты» дешево и безопасно. В настоящем отчете мы сосредоточились на структурных и энергетических аспектах ароматичности, поскольку они наиболее совместимы с другими темами типичных

уроков химии. Для оценки энергии стабилизации ароматических соединений мы предлагаем простую для расчета и понимания энергию стабилизации изомеризации (ISE), популяризированную Паулем фон Раге Шлейером и Франком Пюльхофером около 20 лет назад. В качестве практических примеров мы демонстрируем нашу концепцию с системами на основе бензола и пиридина и предлагаем темы для небольших проектов для студентов и школьников.

Ключевые слова: ароматичность, компьютерно-химические эксперименты, образование

Berechnung von Stabilisierungsenergien für aromatische Moleküle in Schulen -Quantenchemie, ein wertvolles Instrument für den Unterricht und Bildung

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ABSTRACT

Aromatizität gehört zu den fundamentalen Konzepten in der Chemie und wird deshalb auch in nahezu jeder grundlegenden Lehrveranstaltung für organische Chemie besprochen. Leider ist es aber bisher kaum möglich dieses Thema so zu unterrichten, dass die Lernenden aktiviert und zum Selbststudium angeregt werden. Durch den Einsatz von Computerchemie und hier im speziellen von Quantenchemie ergibt sich eine preisgünstige und sichere Möglichkeit für den Lernenden die unterrichteten Konzepte selbst zu überprüfen und selbstständig eigene Experimente auszuführen.

In dieser Arbeit setzen wir den Schwerpunkt auf die strukturellen und energetischen Aspekte des Phänomens „Aromatizität“. Beide Aspekte passen am besten zu den anderen Themen, die in einer typischen Anfängerlehrveranstaltung für organische Chemie besprochen werden. Zur Ermittlung der aromatischen Stabilisierungsenergie greifen wir auf die einfach zu berechnende und ebenso einfach zu verstehende *isomerisation stabilization energy* (ISE) zurück, wie sie vor rund 20 Jahren von Paul von Ragué Schleyer and Frank Pühlhofer als einfaches Konzept für unterschiedlichste Systeme vorgestellt wurde. Das vorgestellte Konzept wird mit praktischen Beispielen an Hand von Benzol- und Pyridinderivaten verdeutlicht; kleine Projekte zum eigenständigen Üben und Forschen der Lernenden werden vorgeschlagen.

Schlüsselwörter: Aromatizität, Computerchemische Experimente, Bildung

Polyphenolic profile of selected varieties of Serbian berries

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ABSTRACT

Selected varieties of strawberries, blackberries, and blueberries grown in Serbia have been analyzed, and their polyphenolic profile was determined by HPLC analysis. The occurrence of phenolic acids, flavonols, and anthocyanins has been examined. Caffeic, *p*-coumaric, ferulic, and ellagic acid were identified and quantified. Six flavonols have been identified in berry samples: quercetin, quercetin-glucoside, quercetin-galactoside, kaempferol, kaempferol-glucoside, and rutin. The following anthocyanins were found in strawberries: pelargonidin-glucoside, pelargonidin-rutinoside, cyanidin-glucoside, and cyanidin-malonylglucoside. Cyanidin-glucoside, cyanidin-rutinoside, and cyanidin-malonylglucoside were found in blackberry samples. The following anthocyanins were found in blueberries: delphinidin-galactoside, delphinidin-glucoside, delphinidin-arabinoside, cyanidin-galactoside, cyanidin-glucoside, cyanidin-arabinoside, petunidin-arabinoside, petunidin-galactoside, peonidin-galactoside, and malvidin-galactoside. Statistical multivariate method - principal component analysis (PCA) was used to classify phenolic acids, flavonols, and anthocyanins according to their contents in berry samples. Cluster analysis (CA) was used to classify samples based on the individual polyphenolics content.

Keywords: *anthocyanins, flavonols, phenolic acids, HPLC, berry fruits, PCA analysis*

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Introduction

Specific aroma, attractive color, and juiciness have made berries one of the world's most popular types of fruits. Given the suitability of the fruits to be consumed either fresh or processed and increasing demands in the world market, berry fruits have been very significant for the Serbian economy.

Berries are poor in sodium but rich in potassium, magnesium, phosphorus, manganese, iron, and zinc (Bagdatlioglu et al., 2010; Guedes et al., 2013; Hakala et al, 2003; Khan et al., 2010), and therefore an excellent food choice for people with hypertension. The presence of copper, nickel, and chromium has been detected in raspberries, blueberries, and strawberries (Grembecka and Szefer, 2013). Other than minerals, berries contain vitamins, and fibers (Zhao, 2007), and are exceptionally rich in polyphenolic compounds (Szajdek & Borowska, 2008; Zhao, 2007). These bioactive compounds participate in the prevention of cardiovascular and neurodegenerative diseases, diabetes, and obesity due to their highly expressed capacity to scavenge free radicals (Coates et al., 2007; Mattioli et al., 2020; Mullen et al., 2002; Paredes-López et al., 2010). Anticancer, antimicrobial, and anti-inflammatory properties were observed *in vitro* and *in vivo* (Nile & Park, 2014). According to Miyake et al. (2012), vision preservation during retinal inflammation is achieved by consuming anthocyanin-rich bilberry extracts. The most significant health benefits of berry fruits are assigned to the presence of polyphenolic compounds. Phenolic acids, anthocyanins, flavonols, and tannins also contribute to the quality, nutritional value, color, and flavor of the fruits (Delgado-Vargas et al., 2000).

Considering such a positive effect on human health, this study aimed to provide a comprehensive analysis and an update on individual phenolic compounds in strawberries, blackberries, and blueberries.

Experimental

Chemicals

Purified water (18 MΩcm), prepared by a MicroMed purification system (TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany) was used to prepare all samples and standards. Methanol and acetonitrile (HPLC grade) were obtained from J.T. Baker (Deventer, The Netherlands), and hydrochloric and formic acids were purchased from Merck® (Darmstadt,

Germany). Kaempferol, malvidin-3-O-glucoside chloride, coumaric acid, ferulic acid, caffeic acid, rutin, quercetin (HPLC grade), delphinidin-3-O-glucoside chloride, pelargonidin-3-O-glucoside chloride and ellagic were purchased from Sigma Aldrich (Steinheim, Germany). Cyanidin-3-O-glucoside chloride and quercetin-3-O- β -glucoside (HPLC grade) were from ChromaDex (Irvine, CA, USA).

Samples

Three cultivars of strawberry fruits, five cultivars of blackberry fruits, and three cultivars of blueberry fruits were collected in parts of Western and Southern Serbia (Table 1). Approximately 500 g of each cultivar was collected and stored in the freezer at -18°C.

Table 1. Cultivars and geographic origin of berry fruits

Fruit	Cultivar	Sample designation	Harvesting period	Geographic origin
Strawberry	Čačanska rana	S1	May	Western Serbia
	Alba	S2	May	Western Serbia
	Senga Sengana	S3	June	Southern Serbia
Blackberry	Čačanska bestrna	Bc1	August	Western Serbia
	Triple Crown	Bc2	August	Western Serbia
	Čačanska bestrna	Bc3	August	Southern Serbia
	Thorn free	Bc4	August	Southern Serbia
	Wild blackberry	Bc5	August	Southern Serbia
Blueberry	Draper	B1	July	Western Serbia
	Huron	B2	July	Western Serbia
	Wild blueberry (bilberry)	B3	August	Southern Serbia

Preparation of extracts

Ultrasound-assisted extraction has been recently reported as a more efficient method for the extraction of anthocyanins and flavonols from berry fruits than conventional solvent extraction (Ivanović et al., 2014). Ultrasound-assisted extraction with acidified 80% methanol (0.01%, v/v HCl) was used to prepare anthocyanin and flavonol-containing extracts from berry samples. Frozen berry fruits were milled in the blender to obtain puree, which was subsequently used for extraction. Briefly, 2.0000 g ± 0.0001 g of each berry sample was weighed, and the flask with a sample was placed into the ultrasonic bath and sonicated for 15 min at 25 °C. After sonication, the

extracts were joined, and the solvent was removed by vacuum evaporation. The extracts were stored in the fridge before analysis.

HPLC analysis

A model 1200 (Agilent Technologies, Santa Clara, California, USA) was used for HPLC analysis. The analytical column was C18 Zorbax Eclipse XDB-C18, 5 μ m, 4.6 \times 150 mm (Agilent Technologies, Santa Clara, California, USA). To identify and determine individual anthocyanins, Agilent-1200 series HPLC with the UV-Vis photodiode array detector (DAD) was used. The column was thermostated at 25 °C. Chromatographic separation was performed in an Agilent-Eclipse XDB C-18 4.6 \times 150 mm column. The mobile phase consisted of aqueous 5% formic acid (eluent A) and 80% acetonitrile/5% formic acid (eluent B). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increases 0-25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25-50% B, from 35 to 40 min gradually increases 50-80% B, and for the last 5 min gradually decreases 80-0% B.

Results and Discussion

Identification of individual polyphenolics was conducted by comparing UV/Vis spectra and retention times of compounds to available standards and literature data. The quantification of phenolic acids was performed based on calibration standards for caffeic, ferulic, and *p*-coumaric acid standards in the concentration interval from 0.1 mg/ml to 1 mg/ml, and for ellagic acid in the concentration interval from 0.2 mg/ml to 1 mg/ml.

The quantification of quercetin (Que), kaempferol (Kaem), and rutin (Rut) were performed based on calibration curves for standards of Que, Kaem, and Rut in the concentration interval from 0.1 mg/ml to 1 mg/ml. Quercetin-glucoside (Que-glu) and quercetin-galactoside (Que-gal) were calculated as Que-equivalents, while kaempferol-glucoside (Kaem-glu) was calculated as Kaem-equivalent.

Quantifications of cyanidin-3-*O*-glucoside (Cy-glu), delphinidin-3-*O*-glucoside (Delp-glu), and malvidin-3-*O*-galactoside (Mal-gal) were performed based on calibration curves for the standards of Cy-glu, Delp-glu, and Mal-gal in the concentration interval from 0.1 mg/ml to 1 mg/ml. Cyanidin-3-*O*-rutinoside (Cy-rut), cyanidin-3-*O*-malonylglycoside (Cy-malglu), and

cyanidin-3-*O*-galactoside (Cy-gal) were calculated as cy-glu equivalents. Delphinidin-3-*O*-galactoside (Delp-gal) and delphinidin-3-*O*-arabinoside (Delp-ara) were calculated as Delp-glu equivalents. Petunidin-3-*O*-arabinoside (Pet-ara), petunidin-3-*O*-galactoside (Pet-gal), and peonidin-3-*O*-galactoside (Peo-gal) were calculated based on the calibration curve for Cy-glu (Nyman and Kumpulainen, 2001). Pelargonidin-3-*O*-glucoside (Pg-glu) and pelargonidin-3-*O*-rutinoside (Pg-rut) were calculated based on the calibration curve for the Pg-glu standard in the concentration interval from 0.1 mg/ml to 1 mg/ml. All the measurements were performed in triplicate.

The content of phenolic acids

The content of phenolic acids in analyzed berry fruits has been given in Table 2. In strawberry samples, caffeic, *p*-coumaric, ferulic, and ellagic acids were identified and quantified. Obtained results for caffeic, *p*-coumaric, and ferulic acid were lower than the literature data (Häkkinen & Törrönen, 2000; Jakobek et al., 2007; Matilla et al., 2006). On the other hand, the content of ellagic acid was higher than that one of Croatian strawberries (Jakobek et al., 2007).

In blackberry samples, *p*-coumaric, ferulic, and ellagic acid were detected, and caffeic acid was detected in one sample of blackberries only (Bc5). The content of ellagic acid in blackberries in our study was lower than reported by Đurić et al. (2014). The main cause may be acidic hydrolysis (2 hours, 85 °C) applied in the mentioned study (Đurić et al., 2014). Namely, the main ellagitannin compounds are sanguin H-6 and lambertianin C. Ellagitannins can be hydrolyzed with acids to release hexahydroxydiphenoyl units, which spontaneously form ellagic acid. Therefore, the quantification of ellagic acid is highly dependent on the hydrolysis procedure (Bobinaite et al., 2012). It has been shown that ellagic acid exhibits anticancer properties in living organisms by modifying the metabolism of toxins absorbed from the environment (Vattem & Shetty, 2005). After oral application, hepatoprotective effect against CCl₄ *in vitro* and *in vivo* has been observed (Singh et al., 1999).

Caffeic and ferulic acid were detected in blueberry samples. The content of ferulic acid is following literature data (Jakobek et al., 2007; Matilla et al., 2006). *p*-Coumaric and ellagic acid were not detected in blueberry samples, and a similar was noted in a study by Häkkinen and Törrönen (2000).

Table 2. Content of phenolic acids in analyzed samples (mg/kg)

Sample	Caffeic acid		<i>p</i> -Coumaric acid		Ferulic acid		Ellagic acid	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
S1	0.741±0.001	0.13	3.47±0.05	1.44	1.95±0.02	1.02	125±3	2.40
S2	0.893±0.003	0.34	1.50±0.03	2.00	1.16±0.01	0.86	65±1	1.54
S3	1.127±0.007	0.62	2.00±0.02	1.00	1.68±0.03	1.78	69±1	1.45
Bc1	n.d.	-	4.28±0.07	1.63	1.30±0.01	0.77	76±1	1.31
Bc2	n.d.	-	3.19±0.03	0.94	1.36±0.05	3.68	85±2	2.35
Bc3	n.d.	-	2.81±0.01	0.35	1.74±0.07	4.02	73±1	1.37
Bc4	n.d.	-	2.27±0.01	0.44	1.92±0.03	1.56	97±1	1.03
Bc5	0.981±0.003	0.31	2.83±0.06	2.12	2.15±0.06	2.79	227±5	2.20
B1	9.21±0.05	0.54	n.d.	-	4.50±0.03	0.67	n.d.	-
B2	9.12±0.09	0.99	n.d.	-	20.7±0.3	1.45	n.d.	-
B3	14.37±0.08	0.56	n.d.	-	26.2±0.5	1.91	n.d.	-

n.d. – not detected

The content of flavonols

As can be seen from Table 3, six flavonols have been identified in analyzed samples: Que, Que-glu, Que-gal, Kaem, Kaem-glu, and Rut, with glycosides of Que being more abundant than glycosides of Kaem. The content of Que in Serbian berries was higher than the one reported for Finnish berries (Häkkinen et al., 1999). According to Mikulic-Petkovsek et al. (2012), glycosides of Que represent 46-100% of total flavonols in different types of berries. Efficient extraction of flavonols from apple samples has been achieved by applying methanol and acidified methanol (Rupashinge et al., 2011). Furthermore, the extraction of flavonols from onion by ethanol, 70% methanol, and subcritical water has been described in the literature (Kim et al., 2014; Ko et al., 2011; Numata & Tanaka, 2011).

Rutin was detected in blackberry and blueberry fruits but not in strawberry samples. There is not much data on the presence of Rut in berry fruits. Obtained results for the content of Rut in blackberries were significantly lower than the available data (Okatan, 2020).

The content of anthocyanins

Four anthocyanins were identified in strawberries: Pg-glu, Pg-rut, Cy-glu, and Cy-malglu (Table 4). The most abundant anthocyanin in all samples was Pg-glu (73.8-80.8%), which agrees with the literature data (da Silva et al., 2007). The content of the other anthocyanins depended on the cultivar of strawberries.

Cy-glu, Cy-rut, and Cy-malglu were found in blackberries (Table 5). The most abundant anthocyanin in all samples was Cy-glu, exceeding 85% in Bc3 and Bc4 and exceeding 90% in Bc1, Bc2, and Bc5 of total anthocyanin content. The presence of these anthocyanins was also detected in blackberries from Chile, France, North Macedonia, and the USA (Fang-Chiang & Wrolstad, 2005).

A large number of individual anthocyanins was detected in blueberries: Delp-gal, Delp-glu, Delp-ara, Cy-gal, Cy-glu, Cy-ara, Pet-ara, Pet-gal, Peo-gal, and Mal-gal. Serbian blueberries manifest a high level of similarity to Slovenian and South Korean blueberries regarding anthocyanin profile (Bae et al., 2015; Trošt et al., 2008). Delp-ara was not detected in sample B1. Glycosides of delphinidin were the most abundant in all samples (33.41-39.05%), followed by glycosides of cyanidin (26.08-28.01%).

According to obtained results, it is evident that each of the berry fruits possessed a very specific anthocyanin profile. Glycosides of pelargonidin were found only in strawberries, while the glycosides of delphinidin, malvidin, peonidin, and petunidin were found only in blueberries. Blackberries contained glycosides of cyanidin exclusively. Specific differences among samples were also observed regarding sugar moieties. Rutinosides and malonylglucosides were found in strawberries and blackberries, while arabinosides and galactosides were exclusively found in blueberries.

The beneficial effect of anthocyanins on human health was the subject of many studies. Delphinidin exhibits an antimicrobial effect against *Staphylococcus aureus* bacteria and acts as an antiphlogistic (anti-inflammatory) and immunosuppressant (Roewer & Broschet, 2013a; Roewer & Broschet, 2013b).

Pelargonidin-3-glucoside, cyanidin-3-glucoside, and delphinidin-3-glucoside, as well as their aglycones, exhibit a strong antioxidant effect in the liposomal system and reduce the formation of malonyl-aldehyde, which occurs as a result of UVB radiation. At the same time,

pelargonidin is the most effective in scavenging hydroxyl radicals, while delphinidin is more effective in inhibiting lipid peroxidation and scavenging superoxide radicals (Tsuda et al., 1996). Anthocyanins exert an anti-angiogenic effect. Angiogenesis represents the growth of new blood vessels from existing capillaries or veins, and endothelial cells play a crucial role in this physiological process. However, disruption of the balance in the body can lead to angiogenesis in cases where it is not desirable (the occurrence of cancer, cardiovascular diseases, retinopathy, and nephropathy caused by diabetes) (Xue et al., 2010). Blueberry, strawberry, and cranberry extracts, rich in anthocyanins, have a suppressive effect on the vascular endothelial growth of human keratinocytes, which is caused by the action of the multifunctional cytokine tumor necrosis factor (TNF) (Roy et al., 2002).

Anthocyanins also have a beneficial effect on the cardiovascular system, exerting a vasorelaxant effect (Bell & Gochenaur, 2006). The antidiabetic effect of pelargonidin-3-galactoside, cyanidin-3-glucoside, and delphinidin-3-glucoside is reflected in the promotion of insulin secretion, reduction of blood glucose levels, and prevention of insulin resistance (Li et al., 2015).

Table 3. Content of flavonols (mg/kg) in analyzed samples

Sample	Que		Que-glu		Que-gal		Kaem		Kaem-glu		Rut	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
S1	21.26±0.5	2.35	3.16±0.03	0.95	32.1±0.1	0.31	3.01±0.01	0.33	3.30±0.02	0.61	n.d.	-
S2	14.37±0.2	1.39	3.33±0.05	1.50	21.9±0.3	1.37	1.77±0.02	1.13	2.58±0.07	2.71	n.d.	-
S3	12.90±0.7	5.43	2.48±0.04	1.61	23.2±0.5	2.16	1.84±0.03	1.63	2.34±0.05	2.14	n.d.	-
Bc1	11.59±0.02	0.43	1.55±0.02	1.29	24.4±0.3	1.23	1.72±0.06	3.49	1.76±0.01	0.57	2.59±0.03	1.16
Bc2	10.14±0.1	0.99	2.44±0.01	0.41	26.8±0.7	2.61	1.69±0.05	2.96	1.95±0.03	1.54	5.61±0.05	0.89
Bc3	8.95±0.01	0.89	1.68±0.01	0.60	24.3±0.2	0.82	1.55±0.03	1.94	1.94±0.03	1.55	4.09±0.07	1.71
Bc4	8.09±0.01	0.12	1.76±0.03	1.70	29.8±0.3	1.01	1.66±0.04	2.41	3.08±0.07	2.27	2.42±0.04	1.65
Bc5	10.45±0.02	0.19	15.72±0.05	0.32	40.1±0.5	1.25	2.74±0.05	1.82	2.37±0.06	2.53	16.4±0.3	1.83
B1	13.99±0.5	3.57	2.32±0.07	3.02	29.8±0.6	2.01	1.58±0.01	0.63	3.05±0.02	0.66	9.2±0.03	0.33
B2	16.47±0.3	1.82	2.65±0.03	1.13	35.8±0.3	0.84	1.82±0.01	0.55	3.30±0.04	1.21	7.2±0.01	0.14
B3	16.17±0.3	1.86	2.69±0.08	2.97	38.6±0.7	1.81	1.86±0.02	1.08	3.40±0.01	0.29	31.1±0.9	2.89

n.d.-not detected

Table 4. Content of individual anthocyanins (mg/kg) in strawberry samples

Sample	Pg-glu		Pg-rut		Cy-glu		Cy-malglu	
	c±SD	RSD (%)	c _{sr} ±SD	RSD (%)	c _{sr} ±SD	RSD (%)	c _{sr} ±SD	RSD (%)
S1	76±1	1.31	4.08±0.03	0.73	2.31±0.04	1.73	11.6±0.1	0.86
S2	84±2	2.38	6.05±0.05	0.83	9.77±0.03	0.31	14.0±0.2	1.43
S3	135±5	3.70	6.51±0.05	0.77	12.4±0.1	0.80	13.3±0.1	0.75

Table 5. Content of individual anthocyanins (mg/kg) in blackberry samples

Sample	Cy-glu		Cy-rut		Cy-malglu	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
Bc1	338±8	2.37	22.9±0.2	0.87	10.84±0.05	0.46
Bc2	336±5	1.49	20.2±0.4	1.98	12.8±0.1	0.78
Bc3	202±4	1.98	13.3±0.1	0.75	9.8±0.1	1.02
Bc4	246±4	1.63	16.7±0.3	1.80	23.8±0.3	1.26
Bc5	438±7	1.60	14.3±0.1	0.70	12.21±0.07	0.57

Table 6. Content of individual anthocyanins (mg/kg) in blueberry samples

Sample	Delp-gal		Delp-glu		Delp-ara		Cy-glu		Cy-gal	
	c±SD	RSD (%)	c _{sr} ±SD	RSD (%)						
B1	67±3	4.48	23.4±0.3	1.28	n.d.	-	24.2±0.5	2.07	29±1	3.45
B2	127±4	3.15	128±2	1.56	69±3	4.35	41±2	4.88	149±4	2.68
B3	157±2	1.27	192±3	1.57	57±1	1.75	160±6	3.75	90±1	1.11

n.d.-not detected

Table 6. Content of individual anthocyanins (mg/kg) in blueberry samples (continued)

Sample	Cy-ara		Pet-ara		Pet-gal		Peo-gal		Mal-gal	
	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)	c±SD	RSD (%)
B1	21.3±0.1	0.47	14.1±0.2	1.42	20±1	5.00	36±1	2.78	35.6±0,2	0.56
B2	26.4±0.1	0.38	111±3	2.70	96±2	2.08	56±1	1.78	26.2±0.1	0.38
B3	80±2	2.50	151±2	1.32	172±3	1.74	71±2	2.82	48±1	2.08

Statistical analysis

Statistical multivariate method - principal component analysis (PCA) was used for the classification of phenolic acids, flavonols, and anthocyanins according to their contents in berry samples. Cluster analysis (CA) was used to classify samples based on the individual polyphenolics content. PCA and CA were performed using a statistical package running on a computer (Statistica 8.0, StatSoft, Tulsa, Oklahoma, USA).

Two significant principal components are extracted based on the Kaiser criterion. The first principal component (PC1) (with an eigenvalue of 13.99) explained 58.28% of the variance, and the second principal component (PC2) (with an eigenvalue of 4.14) explained 17.25% of the variance. The first two PCs are sufficient to explain 75.53% of the pattern variation.

Cy-ara, Cy-gal, Delp-glu, Delp-gal, Delp-ara, Pet-gal, Pet-ara, Peo-gal, caffeic, and ferulic acid were located on the positive side of PC1, and the zero-values of PC2. Mentioned anthocyanins were found in blueberries only. Furthermore, ferulic and caffeic acids were the only phenolic acids found in blueberry samples. Glycosides of pelargonidin (found only in strawberries) were located on the negative sides of both PC1 and PC2 (third quadrant). Strawberries were also characterized by the absence of Rut, which was located in the first quadrant of the diagram (positive sides of PC1 and PC2). Cy-rut was found only in blackberries, and the highest content of Cy-glu was detected in blackberry samples. Both Cy-rut and cy-glu were located in the second quadrant of the diagram (Figure 1).

The cluster analysis was applied to the analyzed berry samples using Ward's method with Euclidian distances as the criterion for forming clusters. Three separate clusters were obtained at $(D_{\text{link}}/D_{\text{max}}) \times 100 < 50$. (Figure 2). The samples of blackberries were grouped in the first cluster. The samples of blueberries B2 and B3 were joined in the second cluster. The third cluster included strawberry samples S1, S2, S3, and one blueberry sample B1. The blueberry sample B1 is characterized by lower Que, Que-gal, and anthocyanin content than samples B2 and B3. Furthermore, the content of ferulic acid in B1 is much lower than in samples B2 and B3 and is closer to the ferulic acid level in strawberry samples. PCA analysis of the samples provided a very similar grouping obtained based on CA (Figure 3). It can be concluded that the dominant factor for grouping the samples was the type of anthocyanins in berry fruits, a direct consequence of the genotype of the plant.

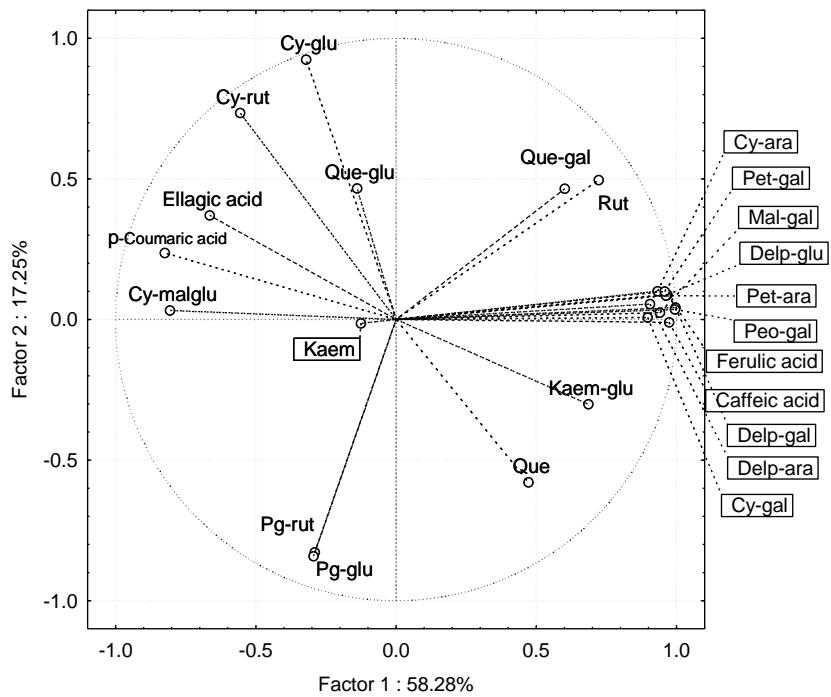


Figure 1. Principal component analysis of individual polyphenolic compounds in berry samples

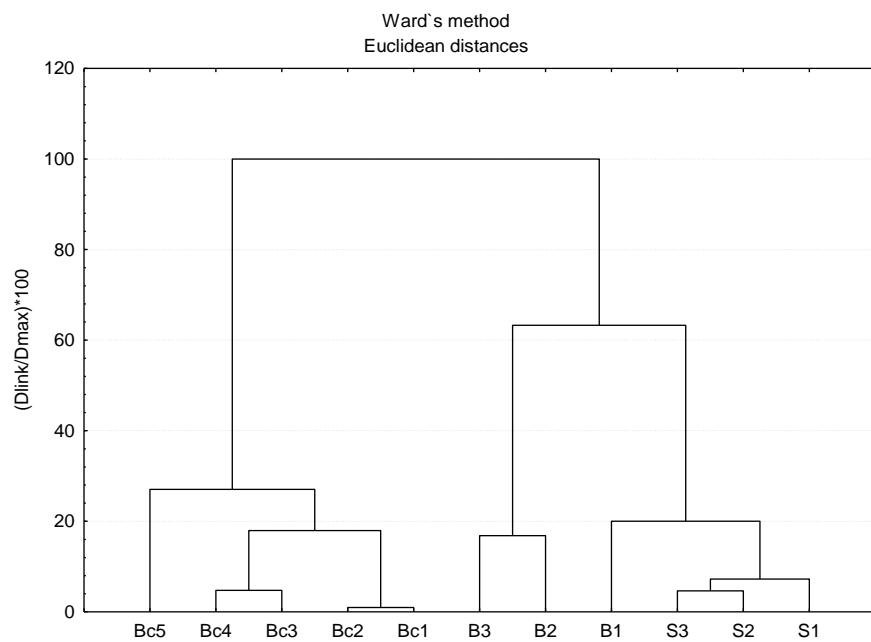


Figure 2. The dendrogram of the cluster analysis of analyzed berries

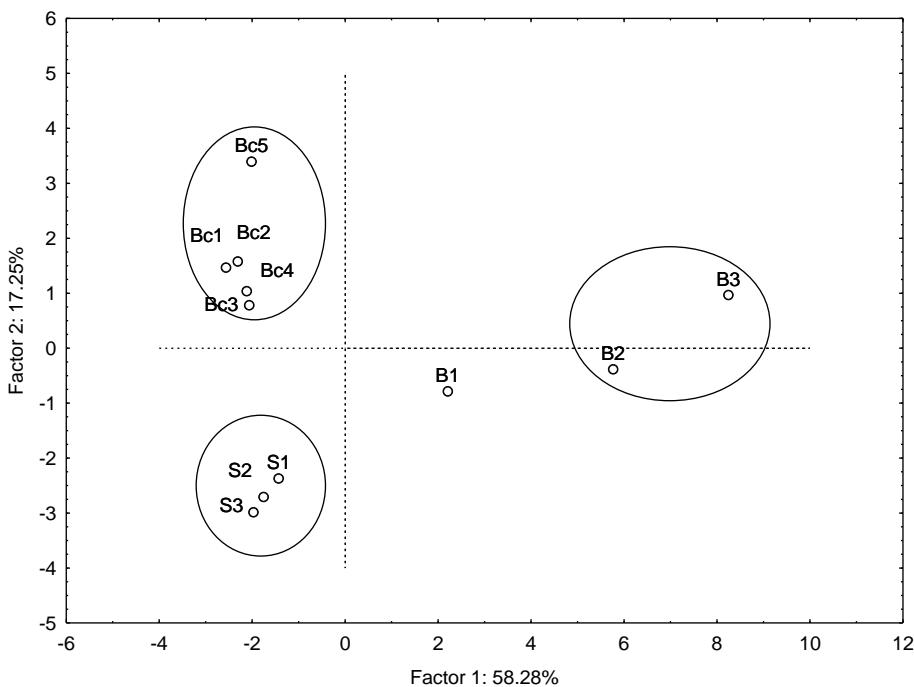


Figure 3. Principal component analysis of analyzed berry samples

Conclusion

Strawberries, blackberries, and blueberries can be considered functional food, regarding the number of bioactive compounds found in these fruits. High similarity in chemical composition and content of phenolic acids, flavonols, and anthocyanins among berries grown in Serbia and the ones grown in other countries has been observed. High amounts of ellagic acid were found in strawberries and blackberries but not in blueberries. The most diverse anthocyanin profile was observed in blueberries, followed by strawberries and blackberries. Nevertheless, certain differences among the cultivars of each fruit have been observed, which can be attributed to the genotype of the plants. The dominant factor for grouping the samples was the type of berry fruit. Based on PCA analysis, individual polyphenolic compounds were grouped in separate quadrants according to the type of berries where those were identified. Cluster analysis of the samples provided a very similar grouping to the one obtained by PCA analysis.

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Conflict-of-Interest Statement

None.

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Polifenolni profil određenih varijeteta srpskih bobica

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SAŽETAK

U radu je ispitana polifenolni profil odabranih sorti jagoda, kupina i borovnica gajenih u Srbiji. Identifikacija i kvantifikacija fenolnih kiselina, flavonola i antocijana je izvršena primenom HPLC analize. Od fenolnih kiselina, u uzorcima su zastupljene kafena, ferulna *p*-kumarna i elaginska kiselina. Od flavonola identifikovani su: kvercetin, kvercetin-glukozid, kvercetin-galaktozid, kemferol, kemferol-glukozid i rutin. U jagodama su identifikovani i kvantifikovani sledeći antocijani: pelargonidin-glukozid, pelargonidin-rutinozid, cijanidin-glukozid i cijanidin-malonilglukozid. U kupinama su identifikovani i kvantifikovani sledeći antocijani: cijanidin-glukozid, cijanidin-rutinozid i cijanidin-malonilglukozid. U borovnicama su identifikovani i kvantifikovani sledeći antocijani: delfnidin-galaktozid, delfnidin-glukozid, delfnidin-arabinozid, cijanidin-galaktozid, cijanidin-glukozid, petunidin-arabinozid, petunidin-galaktozid, peonidin-galaktozid i malvidin-galaktozid. Primenom analize glavnih komponenti (PCA) izvršeno je grupisanje fenolnih kiselina, flavonola i antocijana na osnovu sadržaja u ispitivanim uzorcima. Primenom klaster analize (CA) uzorci jagodastog voća su grupisani u tri klastera na osnovu sadržaja pojedinačnih polifenolnih jedinjenja.

Ključne reči: antocijani, flavonoli, fenolne kiseline, HPLC, bobičasto voće, PCA analiza

Profil polyphénolique de variétés sélectionnées de baies serbes

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RÉSUMÉ

Des variétés sélectionnées de fraises, de mûres et de myrtilles cultivées en Serbie ont été analysées et leur profil polyphénolique a été déterminé par l'analyse HPLC. La présence d'acides phénoliques, de flavonols et d'anthocyanes a été examinée. Les acides caféïques, p-coumarique, férulique et ellagique ont été identifiés et quantifiés. Six flavonols ont été identifiés dans des échantillons de baies : la quercétine, la quercétine-glucoside, la quercétine-galactoside, le kaempférol, le kaempférol-glucoside et la rutine. Les anthocyanines suivantes ont été trouvées dans les fraises : pélargonidine-glucoside, pélargonidine-rutinoside, cyanidine-glucoside et cyanidine-malonylglicoside. Du cyanidine-glucoside, du cyanidine-rutinoside et du cyanidine-malonylglicoside ont été trouvés dans des échantillons de mûres. Les anthocyanines suivantes ont été trouvées dans les myrtilles : delphinidine-galactoside, delphinidine-glucoside, delphinidine-arabinoside, cyanidine-galactoside, cyanidine-glucoside, cyanidine-arabinoside, pétunidine-arabinoside, pétunidine-galactoside, péonidine-galactoside et malvidine-galactoside. La méthode statistique multivariée - l'analyse en composantes principales (ACP) a été utilisée pour classer les acides phénoliques, les flavonols et les anthocyanes en fonction de leur teneur dans les échantillons de baies. L'analyse par grappes (AC) a été utilisée pour classer les échantillons en fonction de la teneur en polyphénols individuels.

Mots-clés : anthocyanes, flavonols, acides phénoliques, HPLC, baies, analyse PCA

Полифенольный профиль отдельных сортов сербских ягод

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АННОТАЦИЯ

Были проанализированы отдельные сорта клубники, ежевики и черники, выращенные в Сербии, и определен их полифенольный профиль с помощью анализа ВЭЖХ. Было исследовано наличие фенольных кислот, флавонолов и антоцианов. Были идентифицированы и количественно определены кофейная, п-кумаровая, феруловая и эллаговая кислоты. В образцах ягод идентифицировано шесть флавонолов: кверцетин, кверцетин-глюкозид, кверцетин-галактозид, кемпферол, кемпферол-глюкозид и рутин. В клубнике обнаружены следующие антоцианы: пелargonидин-глюкозид, пелargonидин-рутинозид, цианидин-глюкозид и цианидин-малонилглюкозид. В образцах ежевики обнаружены цианидин-глюкозид, цианидин-рутинозид и цианидин-малонилглюкозид. В чернике обнаружены следующие антоцианы: дельфинидин-галактозид, дельфинидин-глюкозид, дельфинидин-арабинозид, цианидин-галактозид, цианидин-глюкозид, цианидин-арабинозид, петунидин-арабинозид, петунидин-галактозид, пеонидин-галактозид и мальвидин-галактозид. Для классификации фенолокислот, флавонолов и антоцианов по содержанию их в образцах ягод использовали статистический многомерный метод - анализ основных компонентов (АГК). Кластерный анализ (СА) был использован для классификации образцов на основе содержания отдельных полифенолов.

Ключевые слова: антоцианы, флавонолы, фенолокислоты, ВЭЖХ, ягоды, анализ РСА

Polyphenolisches Profil ausgewählter Sorten serbischer Beeren

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ABSTRACT

Ausgewählte Sorten von Erdbeeren, Brombeeren und Heidelbeeren, die in Serbien angebaut werden, wurden analysiert, und ihr polyphenolisches Profil wurde mittels der HPLC-Analyse bestimmt. Untersucht wurde das Vorkommen von Phenolsäuren, Flavonolen und Anthocyane. Kaffee-, p-Cumar-, Ferula- und Ellagsäure wurden identifiziert und quantifiziert. In Beerenproben wurden sechs Flavonole identifiziert: Quercetin, Quercetin-Glucosid, Quercetin-Galactosid, Kaempferol, Kaempferol-Glucosid und Rutin. Die folgenden Anthocyane wurden in Erdbeeren gefunden: Pelargonidin-Glucosid, Pelargonidin-Rutinosid, Cyanidin-Glucosid und Cyanidin-Malonylglucosid. In Brombeerproben wurden Cyanidin-Glucosid, Cyanidin-Rutinosid und Cyanidin-Malonylglucosid gefunden. Die folgenden Anthocyane wurden in Heidelbeeren gefunden: Delphinidin-Galactosid, Delphinidin-Glucosid, Delphinidin-Arabinosid, Cyanidin-Galactosid, Cyanidin-Glucosid, Cyanidin-Arabinosid, Petunidin-Arabinosid, Petunidin-Galactosid, Peonidin-Galactosid und Malvidin-Galactosid. Das statistische multivariate Verfahren – Hauptkomponentenanalyse (PCA) wurde verwendet, um Phenolsäuren, Flavonole und Anthocyane gemäß ihrem Gehalt in den Beerenproben zu klassifizieren. Die Clusteranalyse (CA) wurde verwendet, um die Proben auf der Grundlage des individuellen Polyphenolgehalts zu klassifizieren.

Schlüsselwörter: Anthocyane, Flavonole, Phenolsäuren, HPLC, Beerenfrüchte, PCA-Analyse

Sulfonated *Lagenaria vulgaris* shell as a potent biosorbent for copper at low pHs

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ABSTRACT

Lagenaria vulgaris shell is composed of lignocellulosic material with the ability to bind metal cations due to hydroxyl, carboxylic, lactonic, and phenolic groups present in its structure. To introduce a greater amount of sulphonate groups characterized by significant ion-exchanging capacity, oxidation, and successive sulfonation were performed. The efficiency of biosorbent based on chemically modified *Lagenaria vulgaris* shell for Cu(II) ion removal from aqueous solution was studied in batch conditions on various initial pHs. Results showed that removal efficiency (95%) did not change in the pH range from 2.0 to 5.0.

Keywords: sulfonation, *Lagenaria vulgaris* shell, biosorption, Cu(II) ions

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Introduction

Among the chemical industries, those that hold heavy metals are the most dangerous in terms of discharging large amounts of wastewater containing toxic metals that living organisms can adsorb. As members of the food chain, these contaminated organisms can increase the concentration of toxic metals in humans (Barakat, 2011). While some heavy metals (iron, manganese, copper, zinc, etc.) are essential for the proper functioning of the human body, ingesting amounts greater than recommended can lead to significant health problems, even death. Mining, refining ores, electroplating, electrolysis deposition, and anodizing cleaning produce significant amounts of wastewater containing copper. Exposure to increased doses of copper causes weakness, lethargy, anorexia, and damage to the gastrointestinal tract (Theophanides & Anastassopoulou, 2002).

Adsorption is a well-known purification technique used in industrial wastewater treatment. Mineral materials (zeolites, activated alumina, *etc.*), activated carbon, industrial by-products, agro-food industry wastes (biosorbents), polymeric materials, *etc.*, can be used as the adsorbents (Akporie & Conradie, 2020; Kurniawan et al., 2005). The adsorption process is influenced by pH, temperature, adsorbent dose, and contact time (Das et al., 2008). The main advantages of this technique are simple equipment, a wide variety of target contaminants, and effectiveness with fast kinetics. But disadvantages such as the high cost of materials (activated carbon is the most used), usual requirements for several types of adsorbents, and rapid saturation that requires expensive regeneration followed by a loss of materials make adsorption economically non-feasible for some chemical industries (Crini & Lichtfouse, 2019).

Natural substances that occur as agricultural, or food-industry waste have been widely studied in the past two decades for wastewater treatment. This process, called biosorption, became popular due to its efficiency and environment-friendly characteristics. The biosorption has found an application in removing heavy metals, phenols, dyes, and other organic pollutants from wastewater. It is a proven alternative to current technologies for the efficient removal of low concentrations of metal ions from aqueous solutions due to cost-effectiveness, simplicity, and high capacity for removing inorganics such as organic pollutants (Karaouzas et al., 2021).

Cellulose is the most abundant polysaccharide on the earth. It is the main structural component of plants and seaweed cell walls and is mainly obtained from the forest, agricultural, industrial, and animal waste. The biomass obtained from all these sources has three main components: cellulose, hemicellulose, and lignin, which content highly depend on the obtained source (Bezerra et al., 2015). Despite the fact that cellulose has many hydroxyl groups which can be complex with metal ions, it has no good adsorption properties. But chemical modifications on cellulosic material surface introducing reactive agents improve its ability to adsorb contaminants from an aqueous medium. The oxidation of cellulose by sodium periodate followed by the sulfonation reaction is a well-known procedure for improving the dry and wet tensile strength and water absorbency and solubility properties of cellulose (Rajalaxmi et al., 2010; Shet & Wallajapet, 1997; Zhang et al., 2008). Nowadays, this procedure was also used for improving the adsorption of heavy metals from water solutions (Suopajarvi et al., 2015). Our attempts with paper pulp showed that this material is not practical because it is challenging to remove swelled cellulose from water. On the other hand, oxidized and successive sulfonated cotton fibers could not reach significant adsorption capacity for copper ions. Therefore, we used the shell of *Lagenaria vulgaris* as starting lignocellulosic material.

Experimental

Materials

The *Lagenaria vulgaris* fruit used in this study was harvested in the vicinity of Leskovac, Serbia, where it grows in controlled conditions without using fertilizers. The fruit sample was washed with deionized water, air-dried in sunlight, cut into 5 cm pieces, and ground using a crusher mill (Waring 8010 ES, Germany). Then it was dried at 55 °C for 24 h and sieved to fractionize particle sizes ranging from 0.8-1.25 mm using successive sieving.

Sodium periodate was purchased from Merck (Germany) and sodium bisulfite was purchased from Sigma–Aldrich (Germany). Copper(II) sulfate was obtained from Zorka (Serbia). All chemicals were of p.a. purity grade, and all solutions were prepared using deionized water.

Preparation of biosorbent

The native biomass (lignocellulosic substrate) was oxidized by soaking 3.00 g biomass in 200 mL of NaIO₄ (16.4 mmol) in the absence of light. Periodate ions react with the cellulose without destroying its fibrous nature and result primarily in the conversion of cellulose C2 and C3 secondary hydroxyl groups to dialdehyde groups (Kim et al., 2000; Kim et al., 2004). The suspension was heated at 55 °C because, at temperatures below 20 °C, the reaction proceeds too slowly, and at temperatures greater than 55 °C, periodate decomposes and the reaction is too fast, causing non-uniformity of the product (Shet, 1996). Different reaction time (3h, 5h, and 24h) was used to enhance dialdehyde content. Sulfonation was carried out by adding the dialdehyde intermediate to 0.1M sodium bisulfite solution and stirred for 72h at room temperature. After filtration, this material was washed with deionized water until the pH reached a constant value close to neutrality and dried at 30 °C.

Characterization of modified material

The biosorbent surface functional groups were characterized using FTIR BOMEM MB-100 (Hartmann & Braun) spectrometer in the range of 4000-400 cm⁻¹. Samples of 100 mg KBr disks containing 1% of finely ground powder (<20 µm) of sulfonated *Lagenaria vulgaris* shell were stored in a vacuum dryer before recording. The point zero charges (pHpzc) and native pH of suspension (pHsus) of the biosorbent were determined using methods described earlier in Stanković et al. (2016).

The batch adsorption studies

Copper removal from model wastewater was investigated using a batch adsorption process. The adsorption experiments were conducted in 250 mL Erlenmeyer flasks containing 125 mL of 50.0 mg/L Cu(II) model solution for 120 min. To maintain consistent experimental conditions, all experiments were carried out using a thermostatic orbital shaker at a shaking speed of 220 rpm. Aliquots of solutions (4.0 mL) were withdrawn at preset time intervals, filtrated, and the filtrates were analyzed for Cu(II) using a flame atomic adsorption spectrometer AAnalyst 300 (Perkin Elmer, USA).

The effect of initial copper concentration on the sorption performance of sulfonated *Lagenaria vulgaris* shell was investigated by immersing 0.5 g of biosorbent in 125 mL solution with initial Cu(II) ions concentrations of 20.0, 50.0, 100.0, 200.0, and 400.0 mg/L at 293 K and at initial solution pH value of 5.0 ± 0.2 during 120 min. The influence of pH on copper removal was

studied by adding 4 g/L of the biosorbent at 293 K at varying pH values (1, 1.5, 2, 3, 4, and 5). The initial pH of the solutions was adjusted using 0.1 M HNO₃ and 0.1 M NaOH solutions. The effect of temperature on copper removal was studied using a solution with initial Cu(II) concentration of 50 mg/L at 10, 20, 35, and 50 °C at fixed initial pH value of 5.0 ± 0.2, biosorbent dose of 4 g/L, and contact time of 120 min.

All the experiments were carried out in duplicates, and the average values were used for further calculations.

The removal efficiency at time t , RE (%), was obtained as follows:

$$RE = (C_0 - C_t)/C_0 * 100\%$$

where C_0 and C_t (mg/L) are the liquid-phase concentrations of Cu(II) ions at initial and any time t , respectively.

Results and Discussion

To determine the optimal time of the oxidation reaction, three systems were performed under the same conditions but with varying reaction times (3h, 5h, and 24h). After filtration, the material was washed with deionized water to remove the used oxidant and freeze-dried providing a 96-98% yield. The dialdehyde content was determined using an alkaline dissolving technique which is consistent with the redox reaction named after Cannizzaro (Hofreiter et al., 1931, Wang et al., 2008). It was observed that oxidation during 5h yielded material with the highest dialdehyde content (80%, 36%, and 32% for treatment during 5h, 24h, and 3h, respectively), which means that the material was more active for further modification, in this case, sulfonation.

The FTIR analysis was used to identify the characteristic functional groups present on the biosorbent. The band of medium intensity at about 1735 cm⁻¹ observed on the FTIR spectrum (Fig. 1) may be attributed to C=O stretching vibrations, probably due to aldehyde formation. This peak is also noticeable on the FTIR spectrum of refined *Lagenaria vulgaris* biomass but of much lower intensity (Mitić-Stojanović et al., 2011). The peaks at about 1350 and 1159 cm⁻¹ might be assigned to the stretching vibrations of the S-O (SO₃-H) groups (Ahsan et al., 2018; Yuen et al., 2005), confirming the incorporation of sulphonic groups into the structure of *Lagenaria vulgaris* shell.

Symmetric stretching vibrations of the hydroxyl group of primary alcohols from lignin give a strong band at about 1040 cm^{-1} , such as the symmetric stretching vibration of the S-O ($\text{SO}_3\text{-H}$) group. The strong, broadband in the $3700\text{-}3000\text{ cm}^{-1}$ region corresponds to the stretching vibrations of -OH functional groups. This band originated from numerous alcoholic and phenolic -OH groups in the cellulose and lignin (Iqbal et al., 2009). The relatively weak band at $3000\text{-}2800\text{ cm}^{-1}$ indicates C-H stretching vibrations in $-\text{CH}$ and $-\text{CH}_2$ groups in the lignin, while the band at 1250 cm^{-1} could be due to stretching C-O vibration.

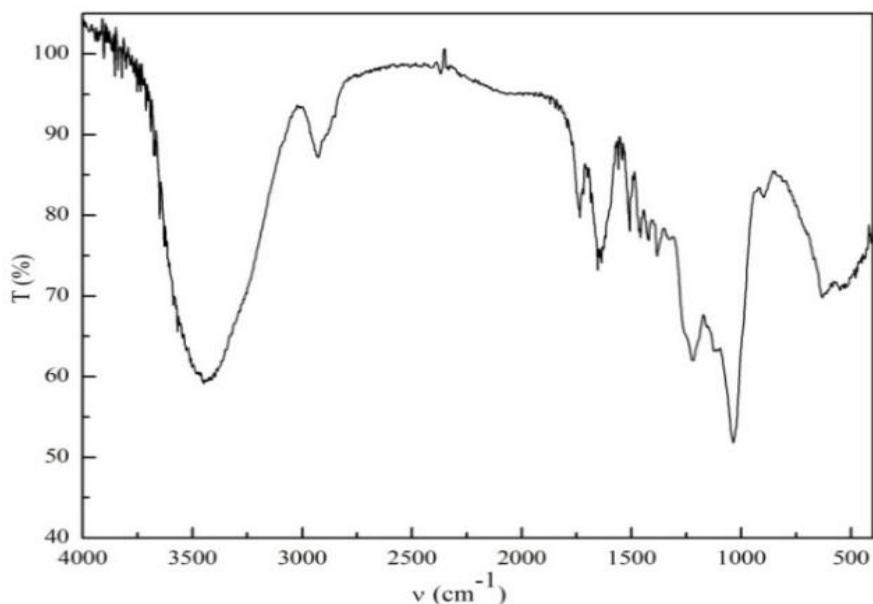


Figure 1. The FTIR spectrum of sulfonated *Lagenaria vulgaris* shell

Within the characterization of the material, the native pH of the suspension (2.78) was determined, as well as the point of zero charges, pH_{pzc}, of the material (3.76). The latter is widely used in characterizing the ionization behavior of a surface. Thus, based on pH_{pzc}, the surface of the biosorbent is expected to be positively charged at low pH values, which indicates that the removal of copper from the model solution is not only a consequence of electrostatic interactions and ion exchange. It can be assumed that complexation with incorporated sulphonic groups plays a significant role in removing Cu(II) ions. It is worth mentioning that the pH_{pzc} value of our material is lower compared to starting material (pH_{pzc} 6.10) or even *Lagenaria vulgaris* shell treated with sulfuric acid (pH_{pzc} 4.95, pH_{sus} 5.65) (Bojić et al., 2016; Ljupković et al., 2011).

Effect of pH

The adsorption experiments focused on studying the influence of solution pH on the removal of copper(II) ions from an aqueous medium. The solution pH is important in the biosorption process and affects not only surface charges and dissociation of functional groups but also solubility, chemical speciation, and diffusion rate of solute (Alothman et al., 2019). Therefore, we investigated the removal efficiency of sulfonated *Lagenaria vulgaris* shell in the pH range of 1.0-5.0 (Fig. 2). It could be assumed that at lower pH, sulphonic groups would be protonated, and competition of H⁺ ions is significant, which would result in poor Cu(II) ions adsorption. But, in this case, the removal efficiency was up to 95% at pH 5.0 and did not change even at pH 2.0. A considerable fall in removal efficiency was observed at pH 1.5 (75%), while at pH 1.0, it reached 23%. Even at very acid conditions (pH 1.5), when the competition of H⁺ ions is significantly strong, the removal efficiency of sulfonated *Lagenaria vulgaris* shell for Cu(II) ions is around 75%. This behavior significantly differs from the starting material, which shows a pronounced dependence of the removal efficiency on the pH values, as seen in Mitić-Stojanović et al. (2011). On the other hand, sulfuric acid-treated *Lagenaria vulgaris* biomass showed comparing results, i.e., variation of pH (in the range from 3-6) caused a small effect on Cu(II) ions sorption onto biosorbent (Ljupković et al., 2011). Authors assumed that the acidic –SO₃ groups on the surface of the biosorbent could not be protonated on investigated pH values, so it does not lead to electrostatic repulsion of positively charged particles that would reduce the ability of copper removal. They also cited the Ozer et al. (2007) suggestion that sulfuric acid reacted with hydroxyl groups in the lignin, forming the sulfuric esters as a non-ionic functional group, which may complex copper(II) cations. Such behavior indicates that this material can be used, with unchanged productivity, to remove copper from wastewater of different sources, without previous pH preparation.

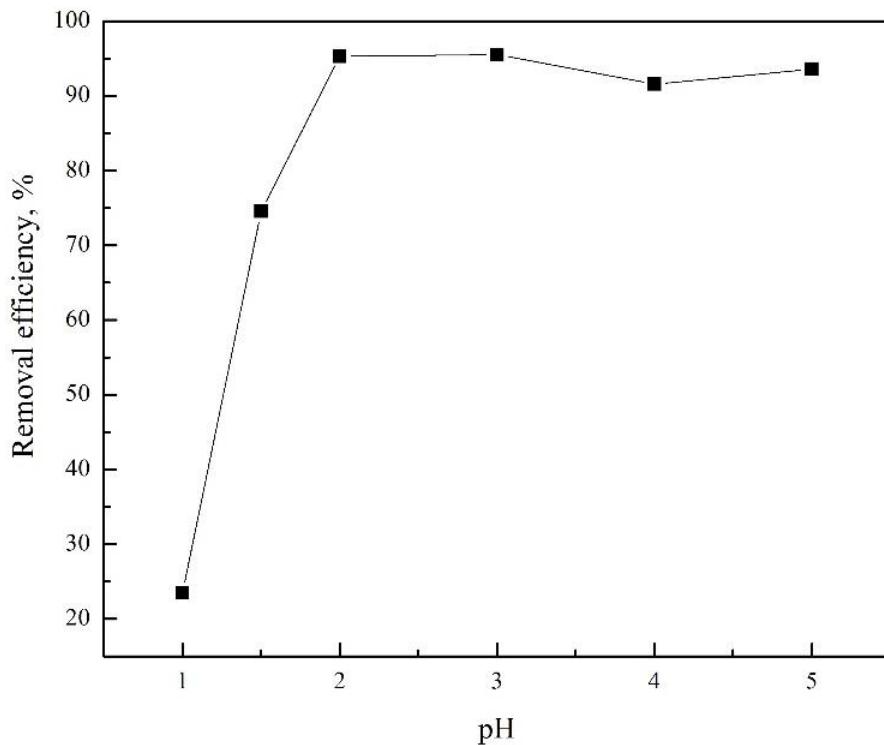


Figure 2. Effect of pH on removal efficiency of sulfonated *Lagenaria vulgaris* shell (Cu(II) conc. 50.0 mg/L, biosorbent dose 4.0 g/L, temp. 20±0.5°C, 120 min)

Effect of initial Cu(II) concentration

Examination of the influence of the initial concentration of copper(II) ions on the removal efficiency of sulfonated *Lagenaria vulgaris* shell, was performed with initial concentrations of Cu(II) ions in the range from 20.0 to 400.0 mg/L in contact with 4.0 g/L of biosorbent granulation 0.8-1.25 mm at 20 ± 0.5 °C and pH 5.0 ± 0.2. As expected, results indicate a decrease in efficiency with increasing concentration of pollutants. At low initial metal ion concentrations, a large number of available, active sites on the adsorbent surface react rapidly and bind all Cu(II) ions present, allowing almost 100% adsorption (94% for initial concentrations of 20.00 and 50.00 mg/L, Fig. 3). On the other hand, at higher concentrations, a larger number of Cu(II) ions remain unabsorbed due to the saturation of a limited number of available, binding sites on the surface of the biosorbent. Contrariwise, the adsorption capacity increased from 4.7 mg/g at an initial Cu(II) concentration of 20.0 mg/L to 76.4 mg/g at an initial Cu(II) concentration of 400.0 mg/L.

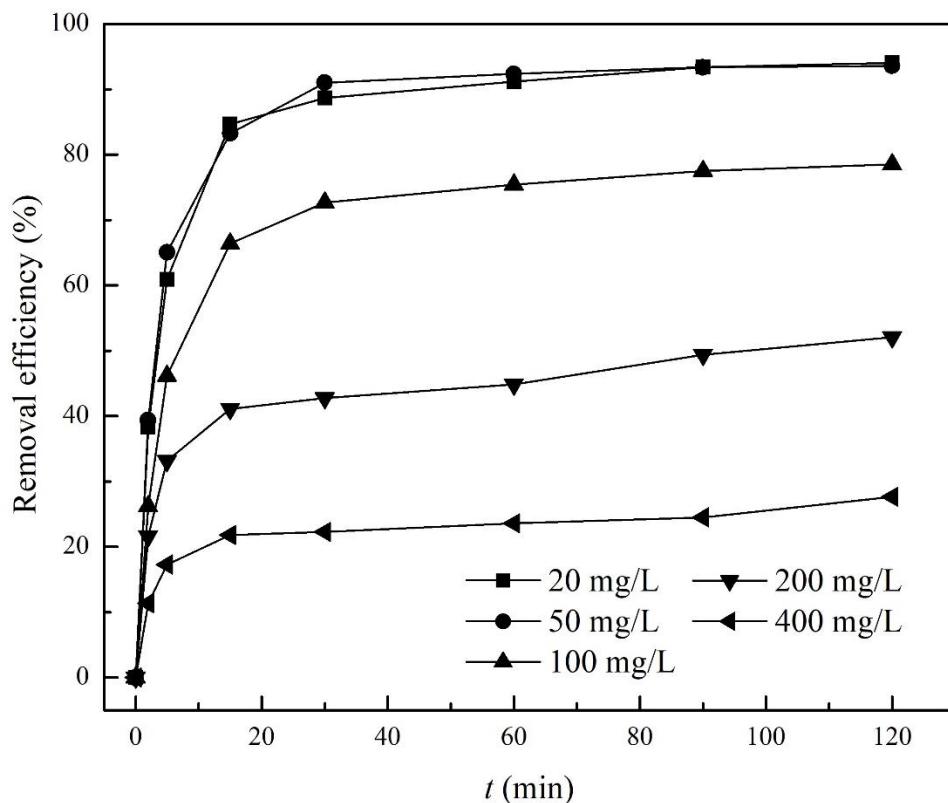


Figure 3. Effect of initial concentration of Cu(II) ions on removal efficiency of sulfonated *Lagenaria vulgaris* shell (biosorbent dose 4.0 g/L, 0.8-1.25 mm sized, pH 5.0 \pm 0.2, temp. 20 \pm 0.5 °C, 120 min).

Effect of initial temperature

The effect of temperature was examined with initial Cu(II) concentrations of 50.00 mg/L in contact with 4.0 g/L of biosorbent sized 0.8-1.25 mm during 120 minutes at 10, 20, 35, and 50 °C. The highest efficiency of copper removal (94%) from the model solution was at 20 °C, while at 10 °C it was 85%. The removal efficiency increases with increasing temperature from 10 °C to 20 °C and then decreases with increasing temperature up to 35 °C, while further heating to 50 °C does not change the removal efficiency. Such behavior suggests that a higher temperature does not favor the removal of copper from the model solution, which might be due to the desorption of copper from the biosorbent surface or adsorbent degradation at higher temperatures. Therefore, the optimal temperature range of copper removal from solution using sulfonated *Lagenaria vulgaris* shell vary between 10-20 °C.

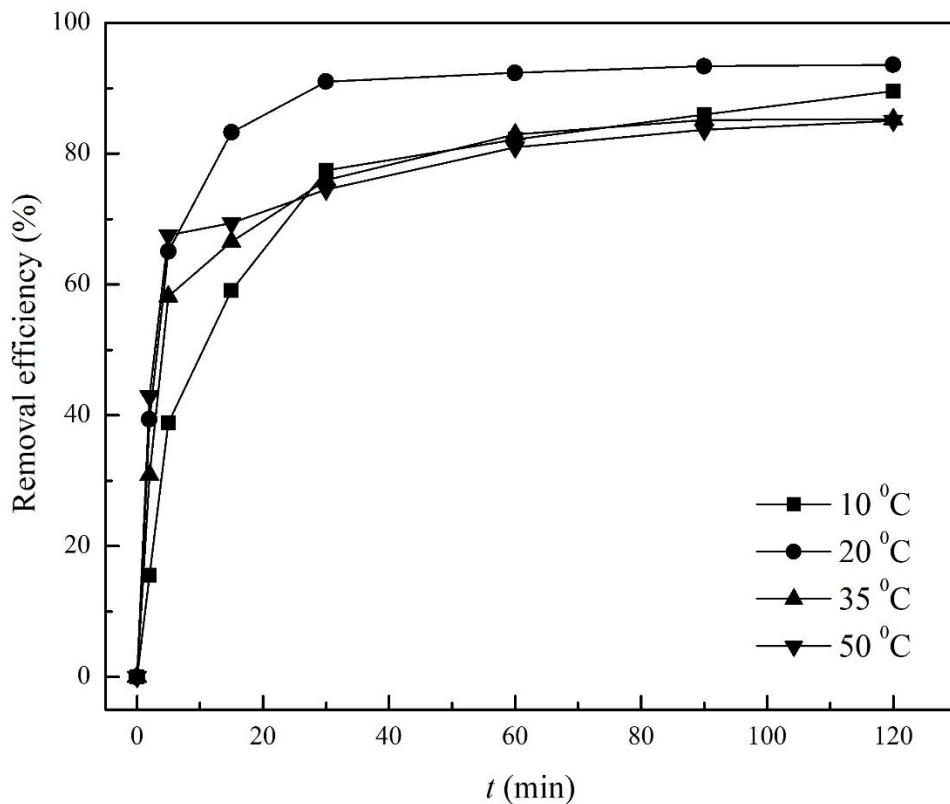


Figure 4. Effect of temperature on removal efficiency of sulfonated *Lagenaria vulgaris* shell (Cu(II) conc. 50.0 mg/L, biosorbent dose 4.0 g/L, pH 5.0, 120 min)

Conclusion

The chemical modification (oxidation and successive sulfonation) of the *Lagenaria vulgaris* shell is very important for the application in the removal of copper ions and other contaminants from aqueous solutions because materials prove new chemical properties that are more approving than the starting materials. These new properties improve the interactions between the modified material and copper ions during adsorption processes in aqueous solutions. Results showed that solution pH does not strongly influence these interactions. Therefore, the sulfonated *Lagenaria vulgaris* shell is promising for removing copper ions from natural and industrial wastewater.

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Conflict-of-Interest Statement

Declarations of interest: none.

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Sulfonovana *Lagenaria vulgaris* ljeska kao potencijalni biosorbent za bakar na niskim pH vrednostima

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SAŽETAK

Ljeska tikve sudovnjače (*Lagenaria vulgaris*) sastavljena je od lignoceluloznog materijala koji pokazuje sposobnost vezivanja metalnih katjona usled prisustva hidroksilnih, karboksilnih, laktonskih i fenolnih grupa u njenoj strukturi. U cilju uvođenja sulfonskih grupa, koje karakteriše značajan jonoizmenjivački kapacitet, izvršena je oksidacija i sukcesivno sulfonisanje lignoceluloznog materijala iz ljeske tikve. Ispitivana je efikasnost biosorbenta na bazi hemijski modifikovane ljeske *Lagenaria vulgaris* za uklanjanje Cu(II) jona iz vodenog rastvora u šaržnim uslovima pri različitim početnim pH vrednostima. Rezultati su pokazali da se efikasnost uklanjanja (95%) nije promenila u pH opsegu od 2.0 do 5.0.

Ključne reči: sulfonacija, *Lagenaria vulgaris* ljeska, biosorpcija, Cu(II) joni

Coquille de *Lagenaria vulgaris* sulfonée comme puissant biosorbant pour le cuivre à faible pH

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RÉSUMÉ

La coquille de *Lagenaria vulgaris* est composée de matière lignocellulosique capable de lier les cations métalliques en raison de présence des groupes hydroxyle, carboxylique, lactonique et phénolique présents dans sa structure. Afin d'introduire une plus grande quantité de groupements sulfoniques caractérisés par une capacité d'échange d'ions importante, une oxydation et une sulfonation successives ont été réalisées. L'efficacité d'un biosorbant à base de coquille de *Lagenaria vulgaris* modifiée chimiquement pour l'élimination des ions Cu(II) d'une solution aqueuse a été étudiée dans des conditions discontinues à différentes valeurs de pH initiales. Les résultats ont montré que l'efficacité d'élimination (95 %) ne changeait pas dans la plage de pH de 2,0 à 5,0.

Mots-clés : sulfonation, coquille de *Lagenaria vulgaris*, biosorption, ions Cu(II)

Сульфированная оболочка *Lagenaria vulgaris* как мощный биосорбент меди при низких рН

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АННОТАЦИЯ

Оболочка *Lagenaria vulgaris* состоит из лигноцеллюлозного материала, обладающего способностью связывать катионы металлов благодаря присутствующим в его структуре гидроксильным, карбоксильным, лактонным и фенольным группам. Для введения большего количества сульфогрупп, обладающих значительной ионообменной способностью, проводили окисление и последовательное сульфирование. Исследована эффективность биосорбента на основе химически модифицированной оболочки *Lagenaria vulgaris* для удаления ионов Cu(II) из водного раствора в стационарных условиях при различных начальных значениях pH. Результаты показали, что эффективность удаления (95%) не изменилась в диапазоне pH от 2,0 до 5,0.

Ключевые слова: сульфирование, оболочка *Lagenaria vulgaris*, биосорбция, ионы Cu(II)

Sulfonierte *Lagenaria vulgaris*-Schale als potenzielles Biosorbens für Kupfer bei niedrigen pH-Werten

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ABSTRACT

Die Schale des Kürbisses (*Lagenaria vulgaris*) besteht aus einem lignozellulosehaltigen Material, das aufgrund der in seiner Struktur vorhandenen Hydroxyl-, Carboxyl-, Lacton- und Phenolgruppen die Fähigkeit besitzt, Metallkationen zu binden. Um eine größere Menge an Sulfonsäuregruppen einzuführen, die durch eine signifikante Ionenaustauschkapazität gekennzeichnet sind, wurden eine Oxidation und eine anschließende Sulfonierung von lignocelluloschem Material aus der Kürbisschale durchgeführt. Die Effizienz des Biosorbens auf der Basis der chemisch modifizierten Schale der *Lagenaria vulgaris* zur Entfernung von Cu(II)-Ionen aus wässriger Lösung wurde unter Batch-Bedingungen bei verschiedenen anfänglichen pH-Werten untersucht. Die Ergebnisse zeigten, dass sich die Entfernungseffizienz (95 %) im pH-Bereich von 2,0 bis 5,0 nicht änderte.

Schlüsselwörter: *Sulfonierung, Lagenaria vulgaris Schale, Biosorption, Cu(II)-Ionen*

Addition effect of *Phoenix canariensis* date and different food by-products on the physicochemical and sensory properties of jelly candy

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ABSTRACT

Jelly candy is an appreciated food for consumers, especially children. Its fortification with functional ingredients could show an interesting effect on human health. This study aimed to define the structural and chemical characteristics, especially that of polyphenolic amounts, of apple candies prepared with different percentages (2, 4, and 6%) of grape by-products (Moscato, Chardonnay, Barbera, and Pinot noir), cocoa bean shell (CBS), coffee silverskin and canary date powder, and to evaluate the consumer overall acceptance of these products. The fortification was achieved by replacing different percentages of apple puree with by-product powders. The jelly candies with the canary date and Pinot noir by-product showed the highest quantities of polyphenolic compounds (6.18 and 4.54mg GAE/g DW, respectively) and the greatest antioxidant capacity (24.04 and 26.54 μ mol TE/g DW, respectively) while the coffee silverskin candy had the lowest values. In general, the use of canary dates and by-product powders in candy production increased hardness, polyphenol and fiber contents, and antioxidant capacity. Sensory analysis showed that the candies obtained with coffee silverskin powder had the least overall liking at 6% substitution. However, no significant differences were recorded between the control and candies at 2 and 4% substitution for all studied functional ingredients.

Keywords: candy, by-product, valorization, polyphenol, antioxidant capacity, *Phoenix canariensis* date

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Introduction

Food by-products are a universal theme of concern as, around the world, the food industry generates each year huge amounts of waste and by-products from a variety of sources. Indeed, these by-products can pose sustainability issues due to the tremendous organic quantities generated in a short period (Coderoni & Perito, 2020). Hence, their disposal would have a negative impact on the environment which differs according to the type of by-product (Grillo et al., 2019; Rojo-Poveda et al., 2019). By focusing on the agricultural sector, fruit processing industries produce by-products of high concentrations of polyphenols and fibers (Mateos-Aparicio et al., 2020) and could represent a potent source of ingredients.

Grape production reached 2018, 77.8 million tons, of which 57% goes to the wine industry (OIV, 2019), where different by-products are produced, such as grape pomace (pulp, leaves, skins, seeds), stems, and wine lees accounting for about 9 million tons generated annually (Gouvinhas et al., 2019). Overall, the volume of waste produced represents a respectable percentage varying between 20-30% of total wine production (Nakov et al., 2020). Wine industry by-products are particularly rich in polyphenols due to poor extraction during winemaking (Maier et al., 2009). Indeed, Cappa et al. (2015) recorded an increase in anthocyanins, flavonols, and procyanidins contents of candies enriched with grape skin powders, which led to an increase in antioxidant activity.

In recent years, the consumption of cocoa and coffee has increased. Consequently, considerable amounts of by-products are generated by the cocoa and coffee industry which creates a significant disposal problem (Grillo et al., 2019). Coffee silverskin has gained attention as a renewable biomass characterized by a rich bioactive composition and promoted applications, especially in the food industry (Gocmen et al., 2019). Moreover, this by-product is a precious natural resource rich in phenolic compounds that may be beneficial to health such as caffeine, caffeoylquinic lactone, and feruylquinic acid (Barbero-López et al., 2020). Cocoa bean shells, also known as husks, are the most important part of the residual materials obtained from the roasting process throughout chocolate production (Barbosa-Pereira et al., 2018). They have been reported to be a considerable source of protein and dietary fiber, with a low-fat content compared to cocoa beans but with a similar profile of volatile compounds (Rojo-Poveda et al.,

2019). This by-product is also rich in antioxidants (mainly flavonoids) and is a potential source of food and beverage ingredients (Grillo et al., 2019).

Phoenix canariensis (*P. canariensis*), commonly named canary dates, are edible but used only for feed as they are astringent. It was used as an ingredient in the biscuit application. Indeed, substituting a percentage of apple puree with canary date powder has given biscuits two-fold fiber and four-fold polyphenolic content compared to biscuits made with wheat flour alone (Turki et al., 2020). Since *P. canariensis* is widely planted and produces a large amount of fruit, there is a great amount of available biomass that can be valorized directly or by recovering its bioactive molecules for use as ingredients in food, pharmaceuticals, and cosmetics.

Considering the different studies analyzed, the beneficial effects that derive from the bioactive compounds of the residues, and the need to reduce the by-product of food companies, the aims of this study were to investigate the effect of the substitution of apple puree by canary date and different by-products powders, at different percentages on the physicochemical characteristics and bioactive, structural and sensory properties of jelly candy and then to choose the best suitable percentage and functional ingredient for the gelatin-based candy production from an organoleptic and chemical-physical point of view.

Experimental

Chemicals

Ethanol ($\geq 99.9\%$), gallic acid (3,4,5-trihydroxy benzoic acid) ($\geq 98\%$), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (97%; Trolox), Folin-Ciocalteu's phenol reagent (2 M), sodium carbonate ($\geq 99.5\%$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were of analytical grade and were purchased from Sigma-Aldrich, Co (Milan, Italy). Ultrapure water was prepared in a Milli-Q filter system (Millipore, Milan, Italy).

Canary date and by-products samples

P. canariensis dates were purchased from "Nizar Jlassi, Aménagement espaces verts" company from the region of Borj El Amri (30 Km, South-west of Tunis, Tunisia). The fruits were washed, manually pitted, and dried in an oven UFE 550 model (Memmert, Schwabach, Germany) at 40 °C with forced air until 5% of moisture was reached, then ground using a ZM200 grinder

(Retsch GmbH, Haan, Germany). Grape marc powders (Moscato, Chardonnay, Barbera, Pinot noir) were provided by Cantine Batasiolo (La Morra, Cuneo, Italy), coffee silverskin by Lavazza SpA and cocoa bean shell by Castagna (Giaveno, Torino, Italy). The powders were stored in vacuum-sealed polyethylene bags at 4 °C until analysis.

Candy preparation

Candies were produced according to Cappa et al. (2015), with several modifications, using sugar, agar, water, apple puree, by-product powder (grape marc powders, coffee silverskin, cocoa bean shell), and canary date powder. In short, the powder of the studied ingredient was first mixed with the water then, sugar, and apple puree was added consecutively by stirring. The resulting mixture was concentrated using a constant temperature cooktop while stirring uniformly to standardize the processing conditions. The concentration process continued until the mixture reached 37 °Brix. The concentrated mixture was then poured into a plate of ten rectangular molds. After 45 minutes of rest at 4 °C, the sweets were extracted from the molds. A preliminary consumer test was performed with 20 consumers (data not shown), and obtained results showed that the maximum percentage acceptable for powder of different studied ingredients was 6%.

According to these results, candies were produced with an aliquot of apple puree replaced by seven different powders in purity to obtain final products with different percentages (2, 4, and 6%) of powders (Table 1). All productions were done in duplicate.

Table 1. Composition (g) of candies with different quantities of studied powders

Ingredients	control	2%	4%	6%
Water	70	70	70	70
Sugar	32	32	32	32
agar	1	1	1	1
Apple puree	67	65	63	61
Studied powder	0	2	4	6

Physico-chemical analysis

The dry matter content of different powders and candies was determined at 105°C using an infrared moisture analyzer (Gibertini Elettronica, Novate Milanese MI, Italy), using 3 g of sample. The water activity of candies was determined at 25±0.02 °C using an AquaLab Pre water activity meter CX-2T (Decagon Devices, Pullman, WA, USA).

The pH and the concentration of soluble solids (°Brix) of candies were measured with a Sension+ pH-meter (HACH, Milan, Italy) and an HI 96801 refractometer (Hanna Instruments, Michigan, USA), respectively.

Color analysis was carried out in transmittance mode on a CM-5 spectrophotometer (Konica Minolta, Tokyo, Japan). L*, a*, and b* CIELab parameters were used to determine the color, where L* is the coefficient of lightness, ranging from 0 black) to 100 (white), a* denotes the colors red and purple (when positive a*) and bluish green (when negative a*), and b* indicates the colors yellow (when positive b*) and blue (negative b*). The difference between the two colors is calculated according to the following formula: $\sqrt{(L_i - L_j)^2 + (a_i - a_j)^2 + (b_i - b_j)^2}$

According to Mokrzycki & Tatol (2011), a standard observer sees the color difference as follows:

- $0 < \Delta E < 1$: observer does not notice the difference
- $1 < \Delta E < 2$: only an experienced observer can notice the difference
- $2 < \Delta E < 3.5$: unexperienced observer also notices the difference
- $3.5 < \Delta E < 5$: clear difference in color is noticed
- $\Delta E > 5$: observer notices two different colors.

All analyses were performed in triplicate.

Textural profile analysis

Textural analyses of candies were carried out by the TA.XT2i Plus Texture Analyser® (Stable Micro System, Godalming, UK) equipped with a 25-kg load cell according to the method described by Bertolino et al. (2011), with slight modifications. For the acquisition of the force-time curve, the Texture Expert Exceed software 2.54 (Stable Micro System, Godalming, UK) was

used. For each matrix, six samples were analyzed. The candy has a trapezoidal shape (Two parallel sides and a line of symmetry) with a height of 25 mm and bases of 40/20 mm and 30/10 mm (length/width).

Candy hardness (N), cohesiveness (adimensional), adhesiveness (mJ), gumminess (N), springiness (mm), chewiness (mJ), and resilience (adimensional) parameters were measured using an SMS P/100 probe (Stable Micro System, Godalming, UK). The texture analyzer setting was as follows: pre-test speed of 1 mm/s, test speed of 1 mm/s, post-test speed of 1 mm/s, a distance of 20%, and a trigger force of 10 g.

Extractions of polyphenols

The extraction of polyphenols was carried out according to the method described by Alahyane et al. (2019) with slight modifications. Briefly, 3 g of the studied powder (or candy) was mixed with 30 mL of ethanol/water solution (80/20, v/v), and the extraction was carried out at 25 °C for 2 h with a VDRL 711 orbital shaker (Asal S.r.l., Milan, Italy) under constant rotatory agitation at 60 rpm. All extracts were centrifuged at 2,800 × g for 10 min at 4°C, and the supernatants were then collected and filtered through a 0.45-µm nylon membrane filter. The samples were kept in amber vials at –18°C. All extractions were performed in triplicate.

Total phenolics content

The total phenolic content (TPC) of the extract was determined based on the Folin–Ciocalteu colorimetric method adapted to a 96-well microplate (Barbosa-Pereira et al., 2018). An aliquot (20 µL) of the sample extract was mixed with 100 µL of Folin-Ciocalteu aqueous reagent (10% v/v) in the wells of a 96-well microplate. After 3 min, 75 µL of a 7.5% sodium carbonate anhydrous solution was added, and the obtained solution was mixed. The solution was allowed to stand for 2 h at 25 °C, and the absorbance was measured at 740 nm against a blank in a BioTek Synergy HT spectrophotometric multi-detection microplate reader (BioTek Instruments, Milan, Italy). All determinations were performed in triplicate. A calibration curve of gallic acid (20–100 mg/L) was constructed to determine the concentration which was expressed in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g).

Antioxidant capacity

The antioxidant capacity of the extracts was evaluated by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH*) radical scavenging method described by Barbosa-Pereira et al. (2018).

All the assays were performed in triplicate in 96-well microplates with the BioTek Synergy HT spectrophotometric multi-detection microplate reader (BioTek Instruments, Milan, Italy). Antioxidant capacity was calculated as the inhibition percentage (IP) of DPPH radical as follows:

$$IP (\%) = ((A_0 - A_{30}) / A_0) \times 100$$

where A_0 is the absorbance of the blank and A_{30} is the absorbance at 30 min.

A standard curve of Trolox was constructed (12.5–300 µM) for the evaluation of the radical-scavenging activity values which were expressed as micromoles of Trolox equivalent per gram of dry weight (µmol TE/g).

Liking test

The sensory test was carried out with 25 adult subjects (females=80%; age range: 18–59 years) who were joined up among the staff and students of the University of Turin. Participants received individual trays with four candy samples and rinsed their mouths with noncarbonated water before beginning the evaluation. Participants tasted the samples according to the tray presentation order, blind, without any information about the innovativeness of the candies to avoid a probable effect of the information on liking scores. Participants rated their liking for appearance, odor, taste, flavor, texture, and overall liking using a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like) according to Lim, (2011). Purchase interest (“Will you buy this candy?”) was also rated on a 7-point scale (1 = absolutely no, 7 = absolutely yes). Candies were distributed in a randomized and balanced order. Participants were asked to rinse their mouths with still water for about 1 min between samples. Each consumer test lasted 10 to 15 min.

Statistical analysis

Physico-chemical data were submitted to a one-way analysis of variance (ANOVA) with Duncan's post hoc test at a 95% confidence level, while the Kruskal-Wallis test was applied for

the statistical analysis of the liking test results. Correlation analysis is performed using Pearson's coefficient. Data analyses were conducted using the Statistica 13.3 software (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

The moisture content and water activity of different candies are shown in table 2. The substitution of apple puree with various percentages of powders reduced significantly the moisture content of candies ($p<0.05$). At the same level of substitution, the candies contained almost the same water content ($p>0.01$). All the water activity values were near 0.955. This similarity was due to the adoption of the same cooking conditions. These values were inferior to those reported by Ventura et al. (2013) who obtained water activity values of about 0.984 for reduced-sugar pomegranate juice jelly prepared using an aqueous extract of pomegranate peels.

The pH values varied depending on powder percentage (Table 2). Except for Pinot noir, the substitution of a percentage of apple puree with grapes marc powder increased the acidity of candies. However, canary date, CBS, and coffee silverskin made the apple candies less acid. In general, the most acid candies are ones prepared with Barbera, Moscato, and Chardonnay.

Regarding the color of obtained candies, it was observed that the different powders affected the color parameters compared to the control sample. This effect increased with the used amount of studied functional ingredients (Table 2). The brightness values (L^*) of the candies varied between 11.69 for the barbera candy and 43.64 for the date candy, the b^* parameter (yellowness) varied from 0.24 for the barbera candy to 38.63 for the date candy, whereas the a^* parameter (redness) varied from 2.78 for the control candy to 13.50 for the CBS candy. By fixing the percentage of substitution, all the candies produced with different studied powders showed significant differences in terms of color ($p<0.05$) which is supported by the high values of ΔE ($\Delta E>2$; data not shown). Then, color differences can be detected with eyes between all different candies at the same substitution level.

Table 2. Physico-chemical characteristics of the candies produced with different percentages of studied functional ingredients

Parameter	Samples	C. Moscato	C. Chardonnay	C. Barbera	C. Pinot noir	C. CBS	C. Silverskin	C. Canary date	Significance
Humidity (%)	0%	56.91 ±0.75 ^b	56.91 ±0.75 ^c	56.91 ±0.75 ^c	56.91 ±0.75 ^b	56.91 ±0.75 ^c	56.91 ±0.75 ^c	56.91 ±0.75 ^{bc}	-
	2%	55.60 ±0.64 ^{bBC}	53.77 ±0.44 ^{bA}	55.90 ±0.34 ^{bcBC}	55.72 ±0.47 ^{bBC}	55.00 ±0.44 ^{cAB}	55.02 ±0.45 ^{bcAB}	57.15 ±0.22 ^{cC}	*
	4%	53.99 ±1.52 ^{abA}	51.54 ±0.70 ^{abA}	53.07 ±0.56 ^{bA}	52.66 ±0.56 ^{aA}	52.62 ±0.53 ^{bA}	53.33 ±0.53 ^{bA}	54.37 ±0.24 ^{abA}	ns
	6%	51.22 ±0.61 ^{aABC}	49.70 ±0.59 ^{aAB}	49.18 ±1.15 ^{aA}	52.46 ±0.48 ^{aC}	48.63 ±0.44 ^{aA}	48.99 ±0.55 ^{aA}	52.21 ±1.06 ^{aBC}	*
Significance		ns	**	**	*	**	**	*	
Water activity	0%	0.96 ±0.00 ^b	0.96 ±0.00 ^a	0.96 ±0.00 ^b	0.96 ±0.00 ^a	0.96 ±0.00 ^a	0.96 ±0.00 ^a	0.96 ±0.00 ^{bc}	-
	2%	0.95 ±0.00 ^{abA}	0.95 ±0.00 ^{aA}	0.96 ±0.00 ^{bAB}	0.96 ±0.00 ^{bBC}	0.96 ±0.00 ^{bBC}	0.96 ±0.00 ^{aBC}	0.96 ±0.00 ^{cC}	**
	4%	0.95 ±0.00 ^{abAB}	0.95 ±0.01 ^{aA}	0.95 ±0.00 ^{abABC}	0.96 ±0.00 ^{abD}	0.96 ±0.00 ^{abCD}	0.96 ±0.00 ^{aBCD}	0.95 ±0.00 ^{bABC}	**
	6%	0.95 ±0.00 ^{aA}	0.95 ±0.00 ^{aA}	0.95 ±0.00 ^{aAB}	0.96 ±0.00 ^{abBC}	0.96 ±0.00 ^{aBC}	0.96 ±0.00 ^{aC}	0.95 ±0.00 ^{aA}	***
Significance		*	ns	ns	ns	ns	ns	**	
°Brix	0%	37.63 ±0.03 ^a	37.63 ±0.03 ^a	37.63 ±0.03 ^a	37.63 ±0.03 ^a	37.63 ±0.03 ^a	37.63 ±0.03 ^a	37.63 ±0.03 ^a	-
	2%	38.00 ±0.40 ^{aA}	37.60 ±0.40 ^{aA}	38.17 ±0.32 ^{aA}	37.23 ±0.35 ^{aA}	37.73 ±0.07 ^{aA}	37.53 ±0.13 ^{aA}	37.13 ±0.73 ^{aA}	ns
	4%	37.17 ±0.35 ^{aA}	37.67 ±0.58 ^{aA}	38.37 ±0.35 ^{aA}	37.33 ±0.18 ^{aA}	37.43 ±0.20 ^{aA}	37.37 ±0.58 ^{aA}	37.30 ±1.60 ^{aA}	ns
	6%	37.93 ±0.19 ^{aA}	37.63 ±0.27 ^{aA}	38.37 ±0.37 ^{aA}	37.70 ±0.50 ^{aA}	37.87 ±0.18 ^{aA}	37.63 ±0.19 ^{aA}	37.90 ±2.30 ^{aA}	ns
Significance		ns	ns	ns	ns	ns	ns	ns	
pH	0%	4.10 ±0.01 ^c	4.10 ±0.01 ^c	4.10 ±0.01 ^d	4.10 ±0.01 ^a	4.10 ±0.01 ^a	4.10 ±0.01 ^a	4.10 ±0.01 ^a	-
	2%	3.99 ±0.02 ^{bB}	4.00 ±0.01 ^{bB}	3.93 ±0.01 ^{cA}	4.09 ±0.03 ^{aC}	4.55 ±0.01 ^{bE}	4.33 ±0.02 ^{bD}	4.37 ±0.01 ^{bD}	***
	4%	3.96 ±0.02 ^{abB}	3.95 ±0.01 ^{aB}	3.89 ±0.02 ^{bA}	4.15 ±0.01 ^{bC}	4.76 ±0.01 ^{cF}	4.49 ±0.01 ^{cD}	4.57 ±0.02 ^{cE}	***
	6%	3.93 ±0.03 ^{aB}	3.94 ±0.01 ^{aB}	3.82 ±0.01 ^{aA}	4.17 ±0.01 ^{bC}	4.95 ±0.02 ^{dF}	4.62 ±0.02 ^{dD}	4.70 ±0.01 ^{dE}	***
Significance		***	***	***	**	***	***	***	

	0%	37.87 ±0.91 ^b	37.87 ±0.91 ^b	37.87 ±0.91 ^b	37.87 ±0.91 ^b	37.87 ±0.91 ^c	37.87 ±0.91 ^c	37.87 ±0.91 ^a	-
	2%	28.21 ±0.40 ^{aE}	27.47 ±0.47 ^{aE}	13.12 ±0.56 ^{aA}	22.61 ±0.27 ^{aD}	21.05 ±0.37 ^{bC}	17.29 ±0.43 ^{bB}	43.42 ±0.35 ^{bF}	***
L*	4%	27.69 ±0.66 ^{aE}	26.30 ±0.47 ^{aE}	11.69 ±0.54 ^{aA}	21.55 ±0.64 ^{aD}	19.62 ±0.48 ^{abC}	16.60 ±0.46 ^{abB}	43.64 ±0.21 ^{bF}	***
	6%	26.97 ±0.31 ^{aE}	26.81 ±0.23 ^{aE}	11.73 ±0.57 ^{aA}	20.91 ±0.57 ^{aD}	18.69 ±0.21 ^{aC}	14.98 ±0.36 ^{abB}	42.78 ±0.42 ^{bF}	***
	Significance	***	***	***	***	***	***	***	
	0%	2.78 ±0.38 ^a	2.78 ±0.38 ^a	2.78 ±0.38 ^a	2.78 ±0.38 ^a	2.78 ±0.38 ^a	2.78 ±0.38 ^a	2.78 ±0.38 ^a	-
	2%	9.29 ±0.20 ^{bC}	8.22 ±0.20 ^{bB}	10.64 ±0.38 ^{cE}	10.43 ±0.13 ^{bDE}	13.50 ±0.18 ^{cF}	4.76 ±0.09 ^{cA}	9.93 ±0.07 ^{bD}	***
a*	4%	9.49 ±0.23 ^{bC}	8.55 ±0.09 ^{BB}	9.81 ±0.42 ^{bcCD}	9.87 ±0.31 ^{bCD}	12.48 ±0.32 ^{bE}	4.00 ±0.18 ^{bA}	10.50 ±0.12 ^{cD}	***
	6%	9.84 ±0.12 ^{bC}	8.92 ±0.17 ^{BB}	9.03 ±0.45 ^{bB}	10.28 ±0.35 ^{bC}	12.47 ±0.17 ^{bD}	4.26 ±0.09 ^{bcA}	12.97 ±0.14 ^{dD}	***
	Significance	***	***	***	***	***	***	***	
	0%	22.97 ±1.34 ^b	22.97 ±1.34 ^b	22.97 ±1.34 ^b	22.97 ±1.34 ^b	22.97 ±1.34 ^b	22.97 ±1.34 ^b	22.97 ±1.34 ^a	-
	2%	16.35 ±0.86 ^{aD}	14.59 ±0.45 ^{aC}	1.27 ±0.15 ^{aA}	10.17 ±0.24 ^{aB}	14.88 ±0.35 ^{aC}	10.19 ±0.29 ^{aB}	38.63 ±0.58 ^{cE}	***
b*	4%	16.96 ±0.60 ^{aE}	15.04 ±0.38 ^{aD}	0.34 ±0.36 ^{aA}	9.06 ±0.41 ^{aB}	13.06 ±0.59 ^{aC}	7.91 ±0.60 ^{aB}	32.31 ±0.77 ^{bF}	***
	6%	17.75 ±0.49 ^{aE}	16.27 ±0.47 ^{aD}	0.24 ±0.21 ^{aA}	9.49 ±0.39 ^{aB}	13.14 ±0.39 ^{aC}	8.97 ±0.30 ^{aB}	33.37 ±0.67 ^{bF}	***
	Significance	***	***	***	***	***	***	***	

C.: Candy; CBS: Cocoa bean shell; Data are mean values ± standard error; ns: not significant; *p<0.05; **p<0.01; ***p<0.001. Data within the same line having different capital letters were significantly different at p<0.05. Data within the same column having different lowercase letters were significantly different at p<0.05.

Texture evaluation showed that generally the hardness of different candies increased significantly as the percentage of canary date or by-product powder added increased (Table 3; $p<0.05$). The highest results in terms of hardness were recorded for candies prepared with grape pomace, particularly Barbera powder. However, the hardness of grape by-products, with the exception of Barbera, decreased at the 6% substitution level. Only the candies developed with CBS showed lower hardness values than the control candy obtained only from the apple puree base. This decrease was also observed on biscuits fortified with CBS powder (Rojo-Poveda et al., 2020). The lower fiber content of the CBS by-product (Table 4) compared to the other powders may explain this difference. Moisture content, pH optimization, and gelling agent dosage and strength help to adjust the texture of soft candies (Altinok et al., 2020).

The adhesiveness of candies was positively correlated with the hardness ($r=0.93$; with 4% substitution). The candies produced with the canary date and CBS powders were characterized by the lowest insoluble fiber content and showed the highest adhesiveness (-0.42 and -0.36 respectively). This result shows a strong correlation between insoluble fiber and adhesiveness ($r=0.82$). This increase could affect the candy quality in the stage of production and consumption (Altinok et al., 2020). Contrary to the hardness results, the CBS candy displayed the highest cohesiveness values, while the cohesion of the other candies decreased by gradually adding a canary date or different by-products. The chewiest candy was the one prepared with silverskin, the richest powder in fiber (Table 4). Ateş & Elmacı (2018) has also recorded an increase in chewiness after preparing cakes with coffee silverskin. The resilience of the candies generally decreased as the percentage of studied powder increased. However, the powder substitution did not affect the candy springiness. Except for springiness and cohesiveness, significant differences in all texture parameters of candies produced with grape by-products were observed by Altinok et al. (2020). The canary date substitution affected the candy's textural properties, especially on hardness, cohesiveness, and adhesiveness. This impact was also recorded after the biscuit dough substitution by canary date powder (Turki et al., 2020). Indeed, several authors have mentioned that fiber addition has an interesting effect on the micro- and macrostructure of jelly candies (Altinok et al., 2020; Cappa et al., 2015).

Table 3. Mean values of texture parameter evaluated on candies prepared with different percentages of studied functional ingredient

Parameter	Samples	C. Moscato	C. Chardonnay	C. Barbera	C. Pinot noir	C. CBS	C. Silverskin	C. Canary date	Significance
Hardness (N)	0%	5.81 ±0.14 ^a	5.81 ±0.14 ^a	5.81 ±0.14 ^a	5.81 ±0.14 ^a	5.81 ±0.14 ^b	5.81 ±0.14 ^a	5.81 ±0.14 ^a	-
	2%	7.08 ±0.18 ^{cD}	7.14 ±0.10 ^{bDE}	7.51 ±0.07 ^{bE}	7.27 ±0.13 ^{cDE}	5.44 ±0.09 ^{aA}	6.32 ±0.10 ^{bC}	5.84 ±0.14 ^{aB}	***
	4%	7.10 ±0.07 ^{cD}	7.34 ±0.20 ^{bDE}	7.39 ±0.15 ^{bDE}	7.61 ±0.13 ^{cE}	5.80 ±0.06 ^{bA}	6.62 ±0.07 ^{cC}	6.22 ±0.07 ^{abB}	***
	6%	6.36 ±0.10 ^{bB}	6.05 ±0.14 ^{aA}	7.75 ±0.13 ^{bD}	6.53 ±0.10 ^{bC}	5.86 ±0.10 ^{bA}	6.86 ±0.05 ^{cC}	6.64 ±0.06 ^{bBC}	***
	Significance	***	***	***	***	*	***	**	
Cohesiveness (-)	0%	0.63 ±0.01 ^c	0.63 ±0.01 ^c	0.63 ±0.01 ^c	0.63 ±0.01 ^c	0.63 ±0.01 ^a	0.63 ±0.01 ^b	0.63 ±0.01 ^b	-
	2%	0.58 ±0.01 ^{bB}	0.58 ±0.01 ^{bB}	0.55 ±0.00 ^{bA}	0.57 ±0.00 ^{bB}	0.65 ±0.00 ^{bC}	0.59 ±0.00 ^{aB}	0.54 ±0.01 ^{aA}	***
	4%	0.56 ±0.00 ^{aC}	0.57 ±0.01 ^{bC}	0.49 ±0.00 ^{aA}	0.57 ±0.00 ^{bC}	0.66 ±0.00 ^{bcE}	0.58 ±0.00 ^{aD}	0.54 ±0.01 ^{aB}	***
	6%	0.55 ±0.00 ^{aC}	0.54 ±0.01 ^{aBC}	0.50 ±0.00 ^{aA}	0.55 ±0.00 ^{aC}	0.67 ±0.00 ^{eE}	0.62 ±0.00 ^{bD}	0.54 ±0.01 ^{aB}	***
	Significance	***	***	***	***	***	***	***	
Adhesiveness (mJ)	0%	-0.23 ±0.03 ^b	-0.23 ±0.03 ^{bc}	-0.23 ±0.03 ^a	-0.23 ±0.03 ^{ab}	-0.23 ±0.03 ^b	-0.23 ±0.03 ^a	-0.23 ±0.03 ^c	-
	2%	-0.18 ±0.01 ^{bD}	-0.18 ±0.01 ^{cD}	-0.27 ±0.02 ^{aAB}	-0.2 ±0.02 ^{bCD}	-0.3 ±0.01 ^{aA}	-0.23 ±0.02 ^{aBC}	-0.28 ±0.02 ^{bcAB}	***
	4%	-0.29 ±0.02 ^{aBC}	-0.28 ±0.02 ^{abBC}	-0.27 ±0.02 ^{aC}	-0.28 ±0.02 ^{aBC}	-0.36 ±0.02 ^{aA}	-0.29 ±0.02 ^{aBC}	-0.31 ±0.01 ^{abAB}	*
	6%	-0.31 ±0.01 ^{aB}	-0.31 ±0.01 ^{aB}	-0.27 ±0.02 ^{aBC}	-0.25 ±0.01 ^{abC}	-0.32 ±0.01 ^{aB}	-0.29 ±0.02 ^{aBC}	-0.42 ±0.02 ^{aA}	***
	Significance	***	**	ns	ns	**	ns	**	
Gumminess (N)	0%	3.64 ±0.09 ^a	3.64 ±0.09 ^b	3.64 ±0.09 ^a	3.64 ±0.09 ^a	3.64 ±0.09 ^{ab}	3.64 ±0.09 ^a	3.64 ±0.09 ^c	-
	2%	4.09 ±0.15 ^{bC}	4.13 ±0.07 ^{cC}	4.16 ±0.04 ^{cC}	4.16 ±0.08 ^{bC}	3.54 ±0.06 ^{aB}	3.70 ±0.06 ^{abB}	3.16 ±0.04 ^{aA}	***
	4%	3.97 ±0.04 ^{bCD}	4.19 ±0.18 ^{cDE}	3.65 ±0.10 ^{aB}	4.32 ±0.08 ^{bE}	3.82 ±0.04 ^{bcBC}	3.87 ±0.05 ^{bBC}	3.34 ±0.04 ^{abA}	***
	6%	0.06 ±3.50 ^{aAB}	3.29 ±0.12 ^{aA}	3.90 ±0.06 ^{bC}	3.61 ±0.07 ^{aB}	3.92 ±0.07 ^{cC}	4.24 ±0.03 ^{cD}	3.55 ±0.09 ^{bcB}	***
	Significance	***	***	***	***	**	***	**	
Cheviness (mJ)	0%	13.84 ±0.48 ^a	13.84 ±0.48 ^b	13.84 ±0.48 ^a	13.84 ±0.48 ^a	13.84 ±0.48 ^a	13.84 ±0.48 ^a	13.84 ±0.48 ^b	-
	2%	14.38 ±1.62 ^{abBC}	15.44 ±0.33 ^{cBC}	16.67 ±0.21 ^{cC}	16.00 ±0.31 ^{bBC}	14.07 ±0.22 ^{aAB}	14.27 ±0.26 ^{abABC}	12.02 ±0.31 ^{aA}	**

	4%	14.25 \pm 1.23 ^{aAB}	15.88 \pm 0.51 ^{cBC}	14.49 \pm 0.35 ^{aAB}	16.91 \pm 0.36 ^{bC}	15.18 \pm 0.16 ^{bBC}	15.09 \pm 0.26 ^{bBC}	13.07 \pm 0.15 ^{abA}	**
	6%	12.10 \pm 1.52 ^{aA}	12.46 \pm 0.45 ^{aA}	15.61 \pm 0.29 ^{bBC}	13.80 \pm 0.20 ^{aAB}	15.86 \pm 0.20 ^{bBC}	16.82 \pm 0.15 ^{cC}	13.96 \pm 0.42 ^{bAB}	***
	Significance	ns	***	***	***	***	***	*	
Springiness (mJ)	0%	3.80 \pm 0.07 ^a	-						
	2%	3.45 \pm 0.35 ^{aA}	3.74 \pm 0.06 ^{aAB}	4.00 \pm 0.03 ^{bB}	3.84 \pm 0.03 ^{aAB}	3.98 \pm 0.08 ^{bB}	3.86 \pm 0.04 ^{abAB}	3.80 \pm 0.08 ^{aAB}	ns
	4%	3.60 \pm 0.31 ^{aA}	3.81 \pm 0.06 ^{aA}	3.98 \pm 0.02 ^{bA}	3.91 \pm 0.02 ^{aA}	4.02 \pm 0.01 ^{bA}	3.9 \pm 0.02 ^{abA}	3.91 \pm 0.01 ^{aA}	ns
	6%	3.48 \pm 0.43 ^{aA}	3.79 \pm 0.04 ^{aA}	4.00 \pm 0.04 ^{bA}	3.83 \pm 0.04 ^{aA}	4.06 \pm 0.04 ^{bA}	3.97 \pm 0.03 ^{bA}	3.93 \pm 0.07 ^{aA}	ns
	Significance	ns	ns	**	ns	*	ns	ns	
Resilience (-)	0%	0.32 \pm 0.00 ^c	0.32 \pm 0.00 ^b	0.32 \pm 0.00 ^{ab}	0.32 \pm 0.00 ^c	0.32 \pm 0.00 ^b	0.32 \pm 0.00 ^c	0.32 \pm 0.00 ^c	-
	2%	0.28 \pm 0.00 ^{bA}	0.28 \pm 0.00 ^{aA}	0.29 \pm 0.00 ^{aA}	0.28 \pm 0.00 ^{bA}	0.31 \pm 0.00 ^{aB}	0.28 \pm 0.00 ^{aA}	0.31 \pm 0.00 ^{cB}	***
	4%	0.28 \pm 0.00 ^{bA}	0.28 \pm 0.01 ^{aA}	0.38 \pm 0.02 ^{cC}	0.27 \pm 0.00 ^{abA}	0.31 \pm 0.00 ^{abB}	0.28 \pm 0.00 ^{aA}	0.29 \pm 0.00 ^{bAB}	***
	6%	0.27 \pm 0.00 ^{aA}	0.27 \pm 0.00 ^{aA}	0.34 \pm 0.02 ^{bcC}	0.27 \pm 0.00 ^{aA}	0.33 \pm 0.00 ^{cC}	0.30 \pm 0.00 ^{bB}	0.27 \pm 0.00 ^{aA}	***
	Significance	***	***	**	***	***	***	***	

C. : Candy; CBS: Cocoa bean shell; Data are mean values \pm standard error; ns: not significant; *p<0.05; **p<0.01; ***p<0.001. Data within the same line having different capital letters were significantly different at p<0.05. Data within the same column having different lowercase letters were significantly different at p<0.05.

The total phenolic content (TPC) and the free radical scavenging activity (RSA) of the different candies are shown in table 5. The candies produced with the canary date and Pinot noir showed the highest values of RSA and TPC, however, the replacement of apple puree with coffee silverskin reduced the values of RSA and TPC in comparison with the candy control. This result could be explained by the values of RSA and TPC of different powders (Table 4). A strong correlation is determined between the different values of powders and candies in terms of RSA and TPC ($r= 0.91$ and 0.97 respectively; with 4% substitution). There was no significant difference between candies prepared with CBS, Muscat, and Chardonnay in terms of RSA and TPC ($p>0.05$).

The substitution of wheat flour with 4% and 6% of grapes pomace powder (Muscat Hamburg variety) increased the TPC of cakes by 2 - 3 times, respectively (Nakov et al., 2020), as well as the enrichment with 4% and 6% of Moscato by-products at the expense of apple puree increased the TPC of candies by the same percentage (Table 5). Similarly to grape marc, the substitution with canary date powder increased the health benefits of candies as shown with biscuits (Turki et al., 2020). The TPC and RSA of candy produced with a canary date were comparable to the values determined by Rivero et al. (2021) for two gelatin candies developed with raspberry powder and orange juice (491.9- 550.8 mg GAE/100 g; 1.60 and 1.82 mmol Trolox/100 g for TPC and RSA, respectively).

Table 4. Fiber and total phenolic (TPC; mg GAE/g dry weight) content and radical scavenging activity (RSA; μmol eq. Trolox/g dry weight) of powder of the different studied functional ingredients

Powders	Total fiber	Soluble fiber	Insoluble fiber	RSA ($\mu\text{mol TE/g DW}$)	TPC (mg GAE/gDW)
Moscato	$44.3 \pm 4.21^{\text{bc}}$	$3.8 \pm 0.33^{\text{a}}$	$40.5 \pm 3.81^{\text{b}}$	$216.16 \pm 18.08^{\text{bc}}$	$38.84 \pm 3.88^{\text{c}}$
Chardonnay	$44.1 \pm 4^{\text{bc}}$	$3.3 \pm 0.28^{\text{a}}$	$40.8 \pm 3.89^{\text{b}}$	$193.88 \pm 24.49^{\text{b}}$	$33.99 \pm 3.92^{\text{bc}}$
Barbera	$44.0 \pm 3.82^{\text{bc}}$	$4.1 \pm 0.36^{\text{a}}$	$39.9 \pm 3.72^{\text{b}}$	$64.04 \pm 0.61^{\text{a}}$	$17.69 \pm 0.25^{\text{ab}}$
Pinot noir	$53.7 \pm 5.23^{\text{cd}}$	$4 \pm 0.35^{\text{a}}$	$49.7 \pm 2.71^{\text{c}}$	$312.99 \pm 27.30^{\text{c}}$	$67.07 \pm 6.90^{\text{d}}$
CBS	$31.2 \pm 2.91^{\text{a}}$	$4.2 \pm 0.34^{\text{a}}$	$27 \pm 2.48^{\text{a}}$	$117.53 \pm 17.97^{\text{ab}}$	$29.95 \pm 4.83^{\text{bc}}$
Silverskin	$61.9 \pm 5.92^{\text{d}}$	$13.1 \pm 0.79^{\text{c}}$	$48.8 \pm 1.59^{\text{c}}$	$19.74 \pm 2.56^{\text{a}}$	$3.11 \pm 0.10^{\text{a}}$
Canary date	$36.88 \pm 3.5^{\text{ab}}$	$8.91 \pm 0.84^{\text{b}}$	$27.97 \pm 2.6^{\text{a}}$	$1211.84 \pm 49.62^{\text{d}}$	$202.09 \pm 6.23^{\text{e}}$
Significance	***	***	***	***	***

Data are mean values \pm standard error; *** $p<0.001$; data, in the same column, with different letters were significantly different at $p<0.05$

Table 5. Total phenolic content (TPC; mg GAE/g dry weight) and radical scavenging activity (RSA; µmol eq. Trolox/g dry weight) of candies produced with different percentages of studied functional ingredients

Parameter	Samples	C. Moscato	C. Chardonnay	C. Barbera	C. Pinot noir	C. CBS	C. Silverskin	C. Canary date	Significance
RSA (µmol TE/g DW)	0%	3.36 ±0.33 ^a	3.36 ±0.33 ^a	3.36 ±0.33 ^a	3.36 ±0.33 ^a	3.36 ±0.33 ^a	3.36 ±0.33 ^a	3.36 ±0.33 ^a	-
	2%	5.13±0.35 ^{bB}	5.43 ±0.08 ^{aB}	4.49 ±0.24 ^{aB}	7.71 ±0.61 ^{bC}	6.65 ±0.20 ^{bC}	2.37 ±0.20 ^{aA}	11.16 ±0.49 ^{bD}	***
	4%	8.08 ±0.41 ^{cBC}	7.93 ±0.57 ^{bBC}	6.55 ±0.26 ^{bB}	14.9 ±0.82 ^{cD}	8.61 ±0.07 ^{cC}	2.95 ±0.36 ^{aA}	20.91 ±0.19 ^{cE}	***
	6%	12.05 ±0.04 ^{dBC}	13.79 ±0.92 ^{cC}	10.13 ±0.64 ^{cB}	24.04 ±1.57 ^{dD}	14.35 ±0.16 ^{dC}	2.54 ±0.02 ^{aA}	26.54 ±1.22 ^{dD}	***
	Significance	***	**	**	***	***	ns	***	
TPC (mg GAE/g DW)	0%	0.94 ±0.08 ^a	0.94 ±0.08 ^a	0.94 ±0.08 ^a	0.94 ±0.08 ^a	0.94 ±0.08 ^a	0.94 ±0.08 ^b	0.94 ±0.08 ^a	-
	2%	1.53 ±0.18 ^{bBC}	1.62 ±0.01 ^{abBCD}	1.30 ±0.03 ^{bB}	1.95 ±0.11 ^{bCD}	2.00 ±0.23 ^{bD}	0.72 ±0.10 ^{abA}	3.41 ±0.12 ^{bE}	***
	4%	2.10 ±0.11 ^{cC}	2.18 ±0.20 ^{bC}	1.72 ±0.07 ^{cB}	3.49 ±0.14 ^{cD}	2.18 ±0.01 ^{bcC}	0.82 ±0.05 ^{abA}	5.49 ±0.10 ^{cE}	***
	6%	2.87 ±0.04 ^{dB}	3.17 ±0.31 ^{cB}	2.47 ±0.14 ^{dB}	4.54 ±0.22 ^{dC}	3.07 ±0.43 ^{cB}	0.63 ±0.01 ^{aA}	6.18 ±0.30 ^{dD}	***
	Significance	**	**	**	***	*	ns	***	

C. : Candy; CBS: Cocoa bean shell; Data are mean values ± standard error; ns: not significant; *p<0.05; **p<0.01; ***p<0.001. Data within the same line having different capital letters were significantly different at p<0.05. Data within the same column having different lowercase letters were significantly different at p<0.0

The sensory effect of functional ingredient powders on candies was assessed with an overall consumer liking and purchase interest (Table 6). No significant difference was observed in liking among samples at 2 and 4% for all the examined parameters (smell, taste, flavor, texture, overall liking, and purchase interest) except the appearance. The candy prepared with Barbera was the most appreciated in terms of appearance, thanks to its characteristic red color. At 6% of substitution, silverskin and canary date candies exhibited the lowest scores for taste, flavor, overall liking, and purchase interest. They were the least appreciated candies due to the bitterness of these powders. This also could be correlated to the high chewiness of silverskin candy, which implies more time and effort required to masticate it (Altinok et al., 2020). The silverskin candy also showed low values for TPC and RSA.

Candies produced with canary date powder showed the best results in terms of total phenolic compounds and antioxidant capacity. It could be valorized using the percentage of substitution accepted by the tasters (4%).

Table 6. Sum of the ranks for each sensory parameter of candies prepared with different percentages of studied functional ingredients and results of the Kruskall-Wallis test

	Samples	Appearance	Odor	Taste	Flavor	Texture	Overall Satisfaction	Purchase
2%	C. Moscato	1284.0 ^{abc}	1243.5 ^a	1452.0 ^a	1401.5 ^a	1491.0 ^a	1364.0 ^a	1369.0 ^a
	C. Chardonnay	1308.5 ^{abc}	1178.5 ^a	1561.5 ^a	1443.5 ^a	1293.0 ^a	1491.0 ^a	1390.0 ^a
	C. Barbera	1759.5 ^c	1668.0 ^a	1640.5 ^a	1513.5 ^a	1280.0 ^a	1429.5 ^a	1429.0 ^a
	C. Pinot noir	1212.0 ^{abc}	1287.0 ^a	1130.5 ^a	1101.5 ^a	1303.0 ^a	1242.5 ^a	1154.5 ^a
	C. CBS	993.5 ^{ab}	1240.5 ^a	957.0 ^a	1014.5 ^a	1331.0 ^a	1156.5 ^a	1234.0 ^a
	C. Silverskin	777.5 ^a	955.0 ^a	930.0 ^a	1081.5 ^a	882.0 ^a	905.0 ^a	984.0 ^a
	C. Canary date	1576.0 ^{bc}	1338.5 ^a	1239.5 ^a	1355.0 ^a	1331.0 ^a	1322.5 ^a	1350.5 ^a
Significance		***	ns	**	ns	ns	ns	ns
4%	C. Moscato	1257.0 ^{ab}	1415.5 ^a	1416.5 ^a	1409.5 ^a	1395.5 ^a	1479.0 ^a	1361.0 ^a
	C. Chardonnay	1058.5 ^{ab}	1118.5 ^a	1275.5 ^a	1323.5 ^a	1291.0 ^a	1185.0 ^a	1105.5 ^a
	C. Barbera	1680.0 ^b	1257.5 ^a	1570.0 ^a	1477.0 ^a	1313.0 ^a	1491.5 ^a	1522.0 ^a
	C. Pinot noir	1485.5 ^{ab}	1367.0 ^a	1520.0 ^a	1509.5 ^a	1349.0 ^a	1439.5 ^a	1583.0 ^a
	C. CBS	1323.5 ^{ab}	1419.5 ^a	1233.5 ^a	1280.5 ^a	1408.5 ^a	1350.5 ^a	1327.0 ^a
	C. Silverskin	1192.0 ^{ab}	1135.5 ^a	812.0 ^a	969.5 ^a	1169.0 ^a	999.5 ^a	1081.0 ^a
	C. Canary date	914.5 ^a	1197.5 ^a	1083.5 ^a	941.5 ^a	985.0 ^a	966.0 ^a	931.5 ^a
Significance		*	ns	*	ns	ns	ns	*
6%	C. Moscato	1155.5 ^{ab}	1242.0 ^a	1540.0 ^b	1687.5 ^c	1310.5 ^{ab}	1557.0 ^a	1502.5 ^{ab}

C. Chardonnay	1001.5 ^{ab}	984.0 ^a	1243.5 ^{ab}	1259.5 ^{abc}	1242.0 ^{ab}	1259.0 ^a	1109.5 ^{ab}
C. Barbera	1687.5 ^b	1378.0 ^a	1467.0 ^b	1475.5 ^{abc}	1283.5 ^{ab}	1458.5 ^a	1388.5 ^{ab}
C. Pinot noir	1351.0 ^{ab}	1300.5 ^a	1586.5 ^b	1586.0 ^{bc}	1285.5 ^{ab}	1537.5 ^a	1577.0 ^b
C. CBS	1378.0 ^{ab}	1629.5 ^a	1471.0 ^b	1185.5 ^{abc}	1537.0 ^b	1391.5 ^a	1457.0 ^{ab}
C. Silverskin	1534.0 ^b	1220.5 ^a	712.0 ^a	798.5 ^a	697.0 ^a	866.0 ^a	1034.0 ^{ab}
C. Canary date	803.5 ^a	1156.5 ^a	891.0 ^{ab}	918.5 ^{ab}	1555.5 ^b	841.5 ^a	842.5 ^a
Significance	**	ns	***	***	**	**	**

C. : Candy; CBS: Cocoa bean shell; ns: not significant; *p<0.05; **p<0.01; ***p<0.001. Sums of ranks within the same column having different letters were significantly different at p<0.05.

Conclusion

In this way, it would be possible to re-evaluate a by-product and have a greater economic gain for companies as well as obtain products with sources of functional compounds (polyphenols, fibers) with important biological activities while providing the consumer with a pleasant product as it turns out it is candy but healthier. Jelly candies with percentages of apple puree substituted with canary date powder exhibited the best results in terms of antioxidant capacity and total phenolic compounds. The valorization of these candies should be done using the percentage of substitution appreciated by the consumers (4%). It will be interesting to study the nutritional effects of candies developed with these functional ingredients.

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Conflict-of-Interest Statement

Declarations of interest: none.

Informed Consent Statement

Informed consent was obtained from all individual participants included in the study.

Human and Animal Rights Statement

Ethical approval: “All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

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Adicioni efekat urme *Phoenix canariensis* i različitih nusproizvoda hrane na fizičko-hemijska i senzorna svojstva žele bombona

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SAŽETAK

Žele bombone su cenjena hrana među potrošačima, posebno decom. Njihovo obogaćivanje funkcionalnim sastojcima može pokazati interesantan uticaj na ljudsko zdravlje. Cilj ovog istraživanja je bio definisanje strukturnih i hemijskih karakteristika, posebno količine polifenola, bombona od jabuke pripremljenih sa različitim procentima (2, 4, i 6%) praha nusproizvoda grožđa (Muskat, Šardone, Barbera i Crni pino), kakao ljske (CBS), srebrnaste folije ljske kafe i urme sa Kanarskih ostrva, i evaluacija celokupne prihvatljivosti ovih proizvoda od strane potrošača. Obogaćivanje je ostvareno zamenom odgovarajućih procenata pirea od jabuke prahom otpadaka. Žele bombone sa otpacima urme sa Kanarskih ostrva i Black pinot su pokazale najviše količine polifenolnih jedinjenja (6.18 i 4.54 mg GAE/g DW, redom) i najviše antioksidativne kapacitete (24.04 i 26.54 µmol TE/g DW), dok su bombone sa srebrnastom folijom ljske kafe imale najniže vrednosti. Generalno, korišćenje praha otpadaka pri proizvodnji bombona povećava tvrdoću, sadržaj polifenola i vlakana, kao i antioksidativni kapacitet. Senzorna analiza je pokazala da bombone sa prahom srebrnaste folije ljske kafe sa 6% zamene imaju najmanju dopadljivost. Međutim, nisu zabeležene značajne razlike između kontrole i bombona sa 2 i 4% zamene za sve otpatke.

Ključne reči: *nusproizvod, valorizacija, polifenol, antioksidativni kapacitet, bombona, Phoenix canariensis urme*

Effet d'addition de la datte *Phoenix canariensis* et différents déchets alimentaires sur les propriétés physico-chimiques et sensorielles des bonbons gélifiés

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RÉSUMÉ

Les bonbons gélifiés sont un aliment apprécié des consommateurs, surtout des enfants. Son enrichissement en ingrédients fonctionnels pourrait montrer un effet intéressant sur la santé humaine. Cette étude visait à définir les caractéristiques structurales et chimiques, notamment celles des quantités des polyphénols dans les bonbons à la pomme préparés avec différents pourcentages (2, 4 et 6 %) de produits dérivés du raisin (Muscat, Chardonnay, Barbera et Pinot Noir), de pelure de fève de cacao (CBS), de parche de café et de poudre de datte des canaris, ainsi que d'évaluer l'acceptation globale de ces produits par les consommateurs. L'enrichissement a été réalisé en remplaçant différents pourcentages de purée de pommes par des déchets en poudre. Les bonbons à la gelée avec les déchets de datte canari et de pinot noir ont montré les quantités les plus élevées de composés polyphénoliques (6,18 et 4,54 mg GAE/g DW, respectivement) et la capacité antioxydante la plus élevée (24,04 et 26,54 µmol TE/g DW, respectivement) tandis que les bonbons de parche de café avaient les valeurs les plus basses. En général, l'utilisation de déchets en poudre dans la production de bonbons a augmenté la dureté, les quantités de polyphénols et de fibres, ainsi que la capacité antioxydante. L'analyse sensorielle a montré que les bonbons obtenus avec de la poudre de la parche de café avaient la moindre appréciation générale à 6% de substitution. Cependant, aucune différence significative n'a été enregistrée entre le groupe témoin et les bonbons à 2 et 4 % de substitution pour tous les déchets.

Mots-clés : sous-produit, valorisation, polyphénol, pouvoir antioxydant, bonbon, datte *Phoenix canariensis*

Влияние добавки финика *Phoenix canariensis* и различных пищевых отходов на физико-химические и органолептические свойства желейных конфет

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АННОТАЦИЯ

Желейные конфеты ценятся потребителями, особенно детьми. Их обогащение функциональными ингредиентами может оказать интересное влияние на здоровье человека. Это исследование было направлено на определение структурных и химических характеристик, особенно количества полифенолов, яблочных конфет, приготовленных с различным процентным содержанием (2, 4 и 6%) побочных продуктов винограда (мускат, шардоне, барбера и черный пино), шелуха какао (CBS), кофейного шелуха в серебряной фольге и фиников с Каннарских островов, а также оценить общее принятие этих продуктов потребителями. Обогащение было достигнуто путем замены разного процентного содержания яблочного пюре порошкообразными отходами. Желейные конфеты с отходами финика и черного пино показали самые высокие количества полифенольных соединений (6,18 и 4,54 мг GAE/g DW соответственно) и самую высокую антиоксидантную способность (24,04 и 26,54 мкмоль TE/g DW соответственно), в то время как кофе конфеты серебристые имели самые низкие значения. В целом, использование порошкообразных отходов в производстве конфет повышает твердость, содержание полифенолов и волокон, а также антиоксидантную способность. Органолептический анализ показал, что конфеты, полученные с кофейным порошком серебристый, имели наименьшую общую привлекательность при замещении 6%. Однако достоверных различий между контролем и конфетами при 2 и 4% замещении всех отходов не зафиксировано.

Ключевые слова: побочный продукт, валоризация, полифенолы, антиоксидантная способность, конфеты, финики *Phoenix canariensis*

Die additive von *Phoenix canariensis* Datteln und Wirkung verschiedener Lebensmittelabfälle auf die physikalisch-chemischen und sensorischen Eigenschaften von Geleebonbons

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ABSTRACT

Geleebonbons sind bei den Verbrauchern, insbesondere bei Kindern, ein beliebtes Lebensmittel. Seine Anreicherung mit funktionellen Inhaltsstoffen kann eine interessante Wirkung auf die menschliche Gesundheit hervorbringen. Das Ziel dieser Forschungsarbeit war es, die strukturellen und chemischen Eigenschaften, insbesondere den Polyphenolgehalt, von Apfelbonbons zu bestimmen, die mit unterschiedlichen Prozentsätzen (2, 4 und 6 %) hergestellt wurden und dies von Traubennebenprodukten (Muscat, Chardonnay, Barbera und Black Pinot, Kakaobohnenschalen (CBS), Kaffeesilberhaut und Datteln von den Kanarischen Inseln und um die allgemeine Verbraucherakzeptanz dieser Produkte zu bewerten. Die Anreicherung erfolgte durch den Ersatz geeigneter Prozentsätze von Apfelpüree durch Abfallpulver. Die Geleebonbons mit den kanarischen Dattelabfällen und dem schwarzen Pinot zeigten die höchsten Mengen an polyphenolischen Verbindungen (6,18 bzw. 4,54 mg GAE/g DW) und die höchste antioxidative Kapazität (24,04 bzw. 26,54 µmol TE/g DW), während die Bonbons mit Kaffeesilberhaut die niedrigsten Werte aufwiesen. Im Allgemeinen erhöht die Verwendung von Abfallpulvern bei der Bonbonherstellung die Härte, den Polyphenol- und Fasergehalt, sowie die antioxidative Kapazität. Die sensorische Analyse zeigte, dass die hergestellten Bonbons, die mit Pulver aus Kaffeesilberhaut mit 6% Substitution, den geringsten Gesamtgeschmack aufwiesen. Es wurden jedoch keine signifikanten Unterschiede zwischen der Kontrolle und Bonbons mit 2% und 4 % Ersatz für alle Abfälle festgestellt.

Schlüsselwörter: Nebenprodukt, Verwertung, Polyphenol, antioxidative Kapazität, Süßigkeiten,
Phoenix canariensis Dattel

GC-MS profile of homemade fruit brandies

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ABSTRACT

This study aimed to analyze volatile congeners in different types of homemade fruit brandies by applying gas chromatography coupled with mass spectrometry (GC-MS). Volatile compounds were analyzed in seven samples, and the number of identified compounds in the studied samples varied between 12 and 35. An enriched volatile profile was determined for the plum samples, whereas the number of identified compounds was significantly reduced in the case of pear and raspberry samples. From a qualitative point of view, brandies obtained from different fruits showed significant differences because only two compounds (furfural and ethyl decanoate) among 60 identified were found to be common to all examined samples. Regarding the class of identified compounds, esters were the most dominant class identified in all samples, with ethyl lactate being the most prevalent compound except the raspberry brandy sample, which was dominated by alcohols, with pentanol being the major compound. The results obtained in this study have shown that brandies from different fruits are very different in both qualitative and quantitative composition of volatile compounds.

Keywords: fruit brandy, chemical composition, volatiles, GC-MS

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Introduction

Fruit brandies are produced by the alcoholic fermentation and distillation of different fruits with or without stones. Unlike other types of spirits, fruit distillates are rich in volatiles due to high amounts of alcohols and esters. Four different groups of aroma compounds can be detected in the fruit brandies: primary aromatic compounds, which originate from the fruit during ripening; secondary aromatic components, formed during alcoholic fermentation; tertiary aromatic compounds, formed during the distillation process; and quaternary aromatic compounds, formed during the maturation process (Tešević et al., 2005). The quality of the brandy depends on the raw material used (Biernacka & Wardencki, 2012; Coldea et al., 2011; Hernandez-Gomez et al., 2005), fruit preparation (Radeka et al., 2008), fermentation (Matijašević et al., 2019; Soufleros et al., 2005), distillation (Arrieta-Garay et al., 2013; Lukić et al., 2011a; Madrera and Alonso, 2012; Matias-Guiu et al., 2016; Spaho, 2017.), and storage (Madrera et al., 2003; Tsakiris et al., 2014).

Rakija is the national drink of Serbia which can be made of almost any fruit, and each fruit has its specific *rakija* name (the name comes from the name of the fruits they are made from). It is interesting that although *rakija* is used for pleasure in the first place, it is also used as a cure in some cases. The most produced brandy in Serbia is *šljivovica* or plum brandy, a registered trademark today and the national drink of Serbia. The *rakija* is transparent and colorless when it is produced. Afterward, it can be stored in wooden barrels or with a piece of wood inside for extra aroma and a golden color.

This study aimed to determine the volatile composition of different homemade fruit brandies by applying gas chromatography coupled with mass spectrometry (GC-MS).

Experimental

Seven samples were analyzed. All the analyzed samples were homemade fruit brandies produced by the traditional method. In short, after the fermentation of the fruit, the brandy was obtained by double distillation. First, a soft distillate of 25 % without separation of fractions, then the second distillation with separation of 10% of the first one and separation of the middle fraction with 40% of alcohol. Four distillates were obtained from stone fruits - plums (R1 and R2), apricots

(R3), and peaches (R4); two distillates were obtained from pome fruits - apples (R5) and pears (R6); and finally, the distillate obtained from berry fruits - raspberries (R7).

Preparation of *rakija* for GC-MS analysis

Eighty milliliters of spirits were mixed with 80 mL of distilled water and 40 mL of CH₂Cl₂ in a 300 mL conical flask. 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 MgSO₄. 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 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 flush for 1.89 min, at 280 °C, with helium pressure of 50 psi. Ionization mode was an electronic impact at 70 eV. The 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 C₈-C₄₀ *n*-alkanes) with the literary values of the retention indices.

Results and Discussion

In the samples subjected to this study, a total of 60 compounds were identified and presented in Table 1. The ethyl esters were the most dominant class of the esters identified in all samples, with ethyl lactate being the most prevalent compound except in the raspberry brandy sample, where it was not even detected. Ethyl esters are produced during fermentation, and their content increases during aging (Silva and Malcata, 1999). This class of compounds contributes to the flavor with a pleasant fruity and flowery smell (Karagiannis and Lanaridis, 2002), so the presence of ethyl esters is beneficial for the spirit (Soufleros et al., 2001; Tešević et al., 2005). On the other hand, higher alcohols occur naturally in alcoholic beverages as by-products of alcoholic fermentation, and they are quantitatively the largest group of the volatile aroma compounds identified in the raspberry brandy sample (70.8%). The most dominant compound in this sample was pentanol with a contribution of 57.6%, while in the samples R2, R3 and R4 pentanol was present in concentrations less than 1% and not even detected in the samples R1, R5, and R6. According to the literature, the characteristic scent of raspberry brandy comes from terpenes of the ionone type (Nikićević et al., 2004; Robertson et al., 1995; Tapani, 1976). If we look at Table 1, it can be noticed that only two compounds (furfural and ethyl decanoate) among 60 identified were found to be common to all examined samples. Regarding the number of identified compounds, with 35 identified components, both plum distillates seem to possess the richest volatile composition, whereas only 12 compounds were found in pear and raspberry distillates. A comparison of the two plum sample compositions showed some qualitative and quantitative differences regarding the identified compounds. The content of esters in "čačanska rodna" distillate was lower than that of the "ranka" sample, while the contents of alcohols and terpenoids were higher. Four compounds were exclusive to "čačanska rodna" distillate: limonene, 2-methoxy-*p*-cresol, 2-phenyl ethyl acetate, and syringaldehyde. On the other hand, "ranka" distillate could be distinguished from the others by the presence of (*E*)-ethyl cinnamate, dodecanoic acid, and ethyl 9-hexadecenoate, which were detected only in this sample. Regarding the major compound, both samples were dominated by ethyl lactate with the contribution of 24.3% in the sample plum "ranka" and 20.1% in distillate obtained from plum "čačanska rodna". Apricot distillate consisted of 30 components, six detected only in this sample (hexyl acetate, diethyl malonate, isopentyl isovalerate, terpinene-4-ol, geranyl acetate, and γ -undecalactone). In peach distillate, 18

compounds were detected and comprised 96.9% of the total. Peach brandy could be distinguished from the other samples because over 90% of the volatiles were ethyl esters (90.9%). A total of 27 components were detected in apple distillate, with 4-ethyl-phenol and 4-ethyl-2-methoxy-phenol that were detected only in this sample. These two compounds were previously detected in wine and beer due to a spoilage yeast *Brettanomyces* (Caboni et al., 2007). Compared to other fruit distillates analyzed in this study, pear distillate was dominated by ethyl lactate (51.9%) and phenyl ethyl alcohol (27.9%) which introduces a pleasant rose aroma.

Let's compare our results with previously published results related to the chemical and sensory characterization of brandy obtained from the "čačanska rodna" plum variety (Popović et al., 2019). It can be noticed that there are some significant differences in the volatile profile of our sample and the sample analyzed in the cited paper. In comparison to the results of Popović et al., where higher alcohols were the dominant class, with 3-methyl-1-butanol being the major compound followed by ethyl acetate, our sample was dominated by ethyl lactate while 3-methyl-1-butanol and ethyl acetate were not even detected. These differences can be explained by Popović et al. analyzing volatile compounds using the headspace method.

Table 1. Chemical composition of fruit brandy volatiles

No	RI	RN	Compound	Content %						
				R1	R2	R3	R4	R5	R6	R7
1	762	760	Isopentyl alcohol	-	-	-	-	-	-	15.4
2	765	762	Pentanol	-	0.9	0.7	0.5	-	-	67.3
3	775	778	Ethyl butanoate	0.3	0.7	2.8	-	0.4	-	-
4	794	798	Ethyl lactate	24.3	20.1	14.1	39.8	29.5	51.9	-
5	810	815	Furfural	0.7	0.8	0.5	0.6	2.6	0.7	4.5
6	828	839	Ethyl 2-methylbutyrate	-	-	-	-	0.5	-	-
7	840	846	2-Methylbutanoic acid	0.5	-	-	0.8	0.5	2.5	-
8	852	858	n-Hexanol	1.8	0.7	1.7	-	6.5	3.4	-
9	861	867	Isopentyl acetate	0.4	0.7	1.1	-	1.4	2.5	-

10	948	955	1,1-Diethoxy-3-methyl-butane	0.3	0.3	-	-	-	-	-	-
11	954	959	Benzaldehyde	7.8	7.2	9.9	0.9	1.6	-	-	-
12	960	968	Ethyl 2-hydroxyisovalerate	0.5	-	-	0.5	0.5	-	-	-
13	995	997	Ethyl hexanoate	0.7	0.6	2.3	-	1.3	0.6	-	-
14	1009	1007	Hexyl acetate	-	-	2.3	-	-	-	-	-
15	1024	1024	Limonene	-	1.3	-	-	-	-	-	-
16	1030	1034	Benzyl alcohol	4.3	14.7	1.2	-	0.8	-	-	-
17	1054	1062	Ethyl 2-hydroxyhexanoate	0.3	0.5	0.4	0.9	0.7	1.4	-	-
18	1067	1067	Diethyl malonate	-	-	3.5	-	-	-	-	-
19	1069	1067	<i>cis</i> -Linalool oxide	0.7	0.3	-	-	-	-	-	-
20	1085	1084	<i>trans</i> -Linalool oxide	0.7	0.8	1.2	-	-	-	-	-
21	1096	1095	Linalool	-	0.2	5.5	-	-	-	-	1.5
22	1100	1100	<i>n</i> -Nonanal	0.3	-	-	-	-	-	-	0.9
23	1101	1103	Isopentyl isovalerate	-	-	2.2	-	-	-	-	-
24	1110	1115	Phenyl ethyl alcohol	1.3	2.6	0.4	-	6.7	27.9	-	-
25	1164	1163	4-Ethyl-phenol	-	-	-	-	1.1	-	-	-
26	1167	1169	Ethyl benzoate	6.2	5.6	5.4	3.9	2.1	-	-	-
27	1173	1170	Octanoic acid	-	-	-	-	-	0.5	-	-
28	1175	1174	Terpinen-4-ol	-	-	0.4	-	-	-	-	-
29	1177	1181	Diethyl succinate	3.2	4.1	0.6	2.5	10.0	1.1	-	-
30	1188	1186	α -Terpineol	0.4	-	2.2	-	-	-	-	0.9
31	1189	1188	2-Methoxy- <i>p</i> -cresol	-	2.1	-	-	-	-	-	-
32	1193	1194	Ethyl octanoate	3.5	2.7	4.0	2.2	5.9	2.8	-	-
33	1225	1246	Benzaldehyde diethylacetal	0.8	1.8	0.3	-	-	-	-	-
34	1241	1243	Ethyl 2-phenylacetate	-	-	-	-	0.4	-	-	-
35	1254	1254	2-Phenyl ethyl acetate	-	0.2	-	-	-	-	-	-
36	1268	1266	Ethyl salicylate	0.4	0.4	-	-	-	-	-	-

37	1276	1280	4-Ethyl-2-methoxy-phenol	-	-	-	-	1.3	-	-
38	1292	1295	Ethyl nonanoate	0.4	0.4	0.2	-	-	-	-
39	1355	1356	Eugenol	1.6	5.1	1.3	2.3	1.4	-	-
40	1370	1364	Decanoic acid	2.1	1.3	-	-	1.6	-	-
41	1377	1376	(E)- α -Ionol	-	-	-	-	-	-	1.9
42	1380	1379	Geranyl acetate	-	-	1.4	-	-	-	-
43	1381	1381	n-Nonanal diethyl acetal	0.8	0.3	-	-	-	-	-
44	1392	1392	Ethyl decanoate	8.0	3.8	6.8	3.8	9.2	1.8	1.2
45	1396	1393	Vanillin	-	0.3	2.2	-	-	-	-
46	1425	1422	(E)- α -Ionone	-	-	-	-	-	-	1.1
47	1463	1465	(E)-Ethyl cinnamate	0.3	-	-	-	-	-	-
48	1464	1465	γ -Decalactone	-	-	12.7	0.9	-	-	-
49	1561	1565	Dodecanoic acid	0.4	-	-	-	-	-	-
50	1570	1569	γ -Undecalactone	-	-	4.7	-	-	-	-
51	1590	1593	Ethyl dodecanoate	4.5	1.9	1.8	1.6	4.3	-	0.8
52	1657	1655	Syringaldehyde	-	0.6	-	-	-	-	-
53	1789	1795	Ethyl tetradecanoate	1.6	0.5	-	0.5	0.5	-	-
55	1967	1977	Ethyl 9-hexadecenoate	0.8	-	-	-	-	-	-
56	1988	1193	Ethyl hexadecanoate	3.7	4.6	-	16.3	2.5	-	-
57	2156	2163	Ethyl linoleate	1.4	2.5	-	8.8	1.2	-	-
58	2162	2173	Ethyl linolenate	1.4	1.8	-	10.1	0.7	-	0.9
60	2820	2833	Squalene	-	-	-	-	-	-	0.9
Number of constituents				35	35	30	18	27	12	12
Total identified				86.4	92.4	93.8	96.9	95.2	97.1	97.3
Alcohols				7.4	18.9	4.0	0.5	14.0	31.3	82.7
Esters				61.9	51.1	48.9	90.9	71.1	62.1	2.9
Others				17.1	22.4	40.9	5.5	10.1	3.7	11.7

Compounds are 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 C₈-C₄₀; RN: NIST Chemistry WebBook Retention indices; -: not detected. Samples: R1-plum “ranka” brandy; R2-plum “čačanska rodna” brandy; R3-apricot brandy; R4-peach brandy; R5-apple brandy; R6-pear brandy; R7-raspberry brandy.

Conclusion

The qualitative and quantitative composition of the tested brandies obtained from plums, apples, pears, peaches, apricots, and raspberries is very different, even in samples obtained from different cultivars of the same species. This composition difference determines the odor and taste of each fruit brand.

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Conflict-of-Interest Statement

All authors declare that they have no conflict of interest.

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GC-MS profil domaćih rakija od voća

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SAŽETAK

Cilj ovog istraživanja je bilo analiziranje isparljivih jedinjenja u različitim tipovima domaćih rakija od voća korišćenjem gasne hromatografije kuplovane sa masenom spektrometrijom (GC-MS). Isparljiva jedinjenja su analizirana u sedam uzoraka, a broj identifikovanih jedinjenja u proučavanim uzorcima je varirao između 12 i 35. Obogaćeni profil isparljivih jedinjenja je nađen u uzorcima od šljiva, dok je broj identifikovanih jedinjenja značajno smanjen u slučaju uzorka od kruške i maline. Sa kvalitativne tačke gledišta, rakije dobijene od različitih vrsta voća pokazivale su značajne razlike zato što se samo dva jedinjenja (furfural i etil dekanoat) nalaze kao zajednička u svim ispitivanim uzorcima. Što se tiče klase identifikovanih jedinjenja, estri su bili najzastupljeniji u svim uzorcima, sa etil laktatom, kao najprisutnijim, izuzev u uzorku rakije od maline, gde su bili dominantni alkoholi, sa pentanolom kao glavnim jedinjenjem. Rezultati dobijeni u ovom istraživanju pokazali su da su rakije od različitih vrsta voća različite kako u kvalitativnom, tako i u kvantitativnom sastavu isparljivih jedinjenja.

Ključne reči: *rakija od voća, hemijski sastav, isparljiva jedinjenja, GC-MS*

Profil GC-MS des eaux-de-vie de fruits artisanales

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RÉSUMÉ

Cette étude visait à analyser les composés volatils dans différents types d'eaux-de-vie de fruits artisanales en appliquant la chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS). Les composés volatils ont été analysés dans sept échantillons et le nombre de composés identifiés dans les échantillons étudiés variait entre 12 et 35. Un profil volatil enrichi a été déterminé pour les échantillons de prune, alors que le nombre de composés identifiés a été significativement réduit dans le cas des échantillons de poire et de framboise. D'un point de vue qualitatif, les eaux-de-vie obtenues à partir de différents fruits ont montré des différences significatives, car seuls deux composés (le furfural et le décanoate d'éthyle) parmi les 60 identifiés se sont révélés communs à tous les échantillons examinés. En ce qui concerne la classe des composés identifiés, les esters étaient la classe la plus dominante identifiée dans tous les échantillons, le lactate d'éthyle étant le composé le plus répandu à l'exception de l'échantillon d'eau-de-vie à la framboise qui était dominé par les alcools, le pentanol étant le composé principal. Les résultats obtenus dans cette étude ont montré que les eaux-de-vie de différents fruits sont très différentes dans la composition qualitative et quantitative des composés volatils.

Mots-clés : *eau-de-vie de fruit, composition chimique, composés volatils, GC-MS*

ГХ-МС профиль домашних фруктовых бренды

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АННОТАЦИЯ

Это исследование было направлено на анализ летучих конгенеров в различных типах домашних фруктовых бренды с применением газовой хроматографии в сочетании с массспектрометрией (ГХ-МС). Летучие соединения были проанализированы в семи образцах, и количество идентифицированных соединений в исследованных образцах варьировало от 12 до 35. Обогащенный профиль летучих был определен для образцов сливы, тогда как количество идентифицированных соединений было значительно снижено в случае груши и малины. С качественной точки зрения коньяки, полученные из разных фруктов, показали существенные различия, поскольку только два соединения (фурфурол и этилдеканоат) из 60 идентифицированных оказались общими для всех исследованных образцов. Что касается класса идентифицированных соединений, сложные эфиры были наиболее преобладающим классом, идентифицированным во всех образцах, при этом этиллактат был наиболее распространенным соединением, за исключением малинового бренди, в котором преобладали спирты, а пентанол был основным соединением. Результаты, полученные в данном исследовании, показали, что коньяки из разных фруктов сильно отличаются как по качественному, так и по количественному составу летучих соединений.

Ключевые слова: бренди фруктовое, химический состав, летучие вещества, ГХ-МС

GC-MS-Profil hausgemachter Obstbrände

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ABSTRACT

Das Ziel dieser Forschungsarbeit war es, flüchtige Verbindungen in verschiedenen Arten von hausgemachten Obstbränden mit Hilfe der Gaschromatographie gekoppelt mit der Massenspektrometrie (GC-MS) zu analysieren. Flüchtige Verbindungen wurden in sieben Proben analysiert, und die Anzahl der identifizierten Verbindungen in den untersuchten Proben variierte zwischen 12 und 35. In den Pflaumenproben wurde eine größere Menge flüchtiger Verbindungen bestimmt, während die Anzahl der identifizierten Verbindungen bei den Birnen- und Himbeerproben deutlich geringer war. In qualitativer Hinsicht wiesen die, aus verschiedenen Früchten gewonnene Brände, signifikante Unterschiede auf, da nur zwei der 60 identifizierten Verbindungen (Furfural und Ethyldecanoat) in allen untersuchten Proben gemeinsam gefunden wurden. In Bezug auf die Klasse der identifizierten Verbindungen waren Ester die vorherrschende Klasse, die in allen Proben identifiziert wurde, wobei Ethyllactat die am häufigsten vorkommende Verbindung war, mit Ausnahme der Himbeerbrandprobe, die von Alkoholen dominiert wurde, wobei Pentanol die Hauptverbindung war. Die in dieser Untersuchung erzielten Ergebnisse haben gezeigt, dass aus verschiedenen Obstsorten hergestellte Obstbrände, sich sowohl in der qualitativen, als auch in der quantitativen Zusammensetzung der flüchtigen Verbindungen unterscheiden.

Schlüsselwörter: Obstbrand, chemische Zusammensetzung, flüchtige Verbindungen, GC-MS

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