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The quality of Slovenian chestnut honey and its specific properties relevant for medical application and functional nutrition

Kakovost slovenskega kostanjevega medu in njegove poznane lastnosti v prid medicinski uporabi in za funkcionalno prehrano

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Abstract: Chestnut honey is well-described in terms of sensory properties, pollen and chemical composition. Specific bitter taste is accompanied with other typical sensory properties derived from its chemical composition, especially in the nectar of sweet chestnut. Compounds from other sources of nectar and honeydew, especially linden, fir and spruce, with smaller amounts from meadow plants, create the specific sensory and chemical properties of Slovene chestnut honey. Based on the chemical composition of the honey, especially the content and proportions of different inorganic ions, it is possible to track the geographical origin of the pasture. Bees contribute significantly to recognized antimicrobial properties of honey by secretion of enzymes and antimicrobial peptides via the food processing glands. When the honey is used for medical purposes, we have to take precautions to avoid microbial and chemical contamination. For the planning of specific use of honey as a medical application we need to explore in detail specific pharmacological properties of single compounds from the chestnut honey and its contribution to the whole activity during wound treatment. In this paper we present a review of most distinct properties of chestnut honey important for its medical application.

Keywords: antimicrobial activity, antioxidant activity, kynurenic acid, honey contamination, melissopalinology, organic honey

Izvleček: Kostanjev med je dobro opisan tako z osnovnimi senzoričnimi lastnostmi, z melisopalinološko analizo in s kemijsko sestavo. Specifičen grenek okus spremljajo še druge tipične senzorične lastnosti, ki pa imajo osnovo v kemijski sestavi, predvsem izvorne medičine na pravem kostanju. Temu se lahko pridružujejo tudi snovi iz drugih virov medenja, predvsem medenja lipe, jelke in smreke, v manjši meri pa travniških rastlin. Geografskega porekla znotraj Slovenije ne moremo zanesljivo opredeliti na osnovi melisopanlinološke analize, lahko pa na specifični kemijski sestavi, predvsem vsebnosti in razmerji med posamezni anorganskimi ioni. K zaznanemu protimikrobnemu delovanju kostanjevega medu pa bistveno prispevajo same čebele z dodajanje encimov in protimikrobnih peptidov. Za uspešno uporabo kateregakoli medu, tudi kostanjevega, v medicinske namene je nujno zagotavljati visoko kvaliteto pridelave brez mikrobnega in kemičnega onesnaženja. Z natančnejšim poznavanjem farmacevtskih učinkov posameznih snovi iz kostanjevega medu k celoviti negi ran lahko načrtujemo specifične pogoje pridelave in uporabe kostanjevega medu za pripravo ustreznih medicinskih pripomočkov.

Ključne besede: antioksidativna aktivnost, ekološki med, kinurenska kislina, pelodna analiza, onesnaženost medu, protimikrobna aktivnost

Introduction

Chestnut honey was always recognized as something special, especially in Europe. Even nonexperts can recognize some of the key properties of the chestnut honey, such as its specific amber color and bitter taste. In folk medicine it is believed that chestnut honey's specific sensory properties might contribute to its healing strength. However, it is even more likely that this belief comes from the healing experience through decades or even centuries. In the recent decades there has been a substantial increase in new scientific data regarding different properties of chestnut honey, especially its chemical composition that supports the healing effects proposed by folk medicine. Chestnut honey has been added to the list of specific honeys that have been registered for medical use in the last twenty years.

General properties of chestnut honey

Chestnut honey is characterized by a reddish-brown, dark amber color, and is usually clear. The taste of chestnut honey is medium sweet, medium to very bitter, and sometimes a slightly acidic or metallic taste may also be present. The odor and aroma are characteristic and intense, reminiscent of chestnut leaves, caramel, burnt sugar, and it can sometimes give off a slight animal note (Bertoncelj et al. 2011a). This type of honey is characterized by a long-lasting aroma, so its sensory acceptability, especially among younger consumers, is poorer. The specific sensory properties of chestnut honey are mainly influenced by compounds present in small quantities, like organic acids, other aromatic compounds, pigments and phenolic compounds.

The main constituents of honey are sugars, mainly fructose and glucose, with small amounts of other sugars (Bogdanov et al. 2004, Korošec et al. 2016b). Within the project of Slovenian honey characterization in the years 2014-2016, fructose, glucose, sucrose, raffinose, turanose, melezitose and maltose were determined in Slovenian chestnut honey samples (Tab. 1). Chestnut honey is characterized by a high fructose to glucose ratio (F/G = 1.5 - 1.6), suggesting that typical chestnut honey usually remains liquid and is not prone to crystallization. Some physicochemical properties of chestnut honey are presented in Tab. 2. Compared to other types of Slovenian honey, chestnut honey is characterized by a high electrical conductivity and pH value, high content of amino acid proline and high activity of enzyme diastase.

The complex composition of honey derives from components present in small amounts, such as elements, enzymes, organic acids, phenolic compounds, proteins, and vitamins, which also contribute to antimicrobial and antioxidant activity of honey. Compared to other types of Slovenian honey (acacia, linden, fir, spruce, multifloral and forest honey), chestnut honey contains more potassium, calcium and manganese (Kropf et al. 2010). The potassium content is very high

Table 1: Sugar content in chestnut honey (N = 15) (Korošec et al. 2016a). Abbreviations: SD, standard deviation; Min, minimum; Max, maximum.
Tabela 1: Vsebnost sladkorjev v kostanjevem medu (N = 15) (Korošec s sod. 2016a). Okrajšave: SD, standardni odklon; Min, minimum; Max, maksimum.

	Sugar content (g/kg honey)						
Statistics	**Fructose**	**Glucose**	**Sucrose**	**Raffinose**	**Turanose**	**Melezitose**	**Maltose**
Mean	413.3	261.3	1.94	7.64	27.7	75.9	44.0
SD	49.8	29.8	1.55	14.57	5.0	34.0	9.1
Min.	336.7	207.7	0.73	0.00	21.2	46.3	30.6
Max.	479.6	303.5	5.00	43.17	37.0	173.3	54.2

Table 2: Results of physicochemical analysis of chestnut honey samples (N = 29) (Bertoncelj et al. 2011a). Abbreviations:SD, standard deviation; Min, minimum; Max, maximum; DN, diastase number.
Tabela 2: Rezultati fizikalno-kemijskih analiz kostanjevega medu (N = 29) (Bertoncelj s sod. 2011a). Okrajšave: SD, standardni odklon; Min, minimum; Max, maksimum; DN, diastazno število.

Statistics	**Water content (g/100 g)**	**Electrical conductivity (mS/cm)**	**pH**	**Free acids (meq/kg)**	**Lactones (meq/kg)**	**Total acids (meq/kg)**	**Proline content (mg/kg)**	**Diastase (DN)**
Mean	15.9	1.61	5.51	13.3	2.5	15.8	558	18.6
SD	0.9	0.24	0.42	4.7	1.6	5.7	108	4.5
Min	13.7	1.05	4.75	7.3	0.0	8.9	390	13.9
Max.	17.7	2.25	6.18	26.0	6.5	32.5	776	31.9

with an average 3590 mg/kg. The ash content in Slovenian chestnut honey is between 0.55 to 1.04 g/100 g, which is on average 25% higher than in honeydew types of honey and almost 20 times higher than in acacia honey. Chestnut honey also contains various phenolic compounds (phenolic acids and flavonoids). Of the phenolic acids, *p*-coumaric, caffeic and cinnamic acid predominate. The main flavonoids detected in chestnut honey are propolis-derived flavonoids: pinocembrin, chrysin, galangin and pinobanksin, as well as kaempferol and apigenin (Bertoncelj et al. 2011b). Phenolic compounds have been proven to act as antioxidants. Moreover, samples of Slovenian chestnut honey exhibit antioxidant activity; the results are comparable to the antioxidant activity of honeydew honey and are related to the color; darker honeys have higher antioxidant activity (Korošec et al. 2016b).

Geographical variability of chestnut honey in Slovenia

Kropf with colleagues (Kropf et al. 2010) analyzed the composition of chestnut honey from different geographical regions of Slovenia (Perko 1998) and evaluated different physicochemical parameters: electrical conductivity, ash content, pH value, contents of total and free acids, lactones, proline, proteins, color parameters L^*, a^* and b^*, specific rotation, elemental content (S, Cl, K, Ca, Mn, Rb), stable carbon and nitrogen isotope ratios ($\delta 13C_{honey}$, $\delta 13C_{proteins}$ and $\delta 15N$). Samples of chestnut honey originate from all four Slovenian natural geographical macroregions. In the Mediterranean region, chestnut honey is rarely represented, so chestnut honey samples from Alpine (n=17), Dinaric (n=12) and Pannonian macroregion (n=8) were included in the comparison of geographical variability. Chestnut honey from the three different Slovenian regions differed in the content of proline, potassium, rubidium and in the color parameters. Chestnut honey from the Pannonian macroregion contained more rubidium, while honey from the Dinaric macroregion was darker in color and contained less proline. Using the method of linear discriminant analysis (LDA) Kropf with colleagues (Kropf et al. 2010) successfully proved the discrimination of chestnut honey samples according to the geographical origin based on the treated parameters, as the first two axes explained the overall variability of the data. Parameters with the major factors in discriminating Slovenian chestnut honeys were the contents of sulfur, ash and potassium, the color parameter L* (lightness) and the ratio of sulfur to calcium (S/Ca).

Melissopalynology of the chestnut honey

In one of our previous studies we attempted to identify the geographical origin of chestnut honey based on pollen analysis of 28 samples (Golob et al. 2008). We were not able to match it. Partial matching was observed only in acacia honey (honey of the black locust, *Robinia pseudacacia*), most likely because of more evident differences in the phenology of melliferous plants and bigger differences in diversity during blooming of black locust in different phytogeographical regions of Slovenia. Regardless of the extraction of a honey by a beekeeper before chestnut blooming, there was still pollen of the black locust present in more than half of the samples We found following types of pollen: *Trifolium repens*, *Tilia* sp. *Plantago* sp., *Asteracea* type J and *Filipendula* sp. in more than half samples and their phenophases match with the blooming of chestnut. Large-leaved linden (*Tilia palthyphyllus*) blooms regularly just before chestnut, but small-leaved linden (*Tilia cordata*) finishes flowering usually at the beginning of the chestnut bloom. Presence in honey samples of the pollen from plants that bloom before chestnut is usually confirmation of partial mix of honey from previous nectar source. Maple (*Acer* sp.) and flowering ash (*Fraxinus ornus*) are also present in more than half the samples (Golob et al. 2008). Pollen analysis of the chestnut honey is mainly useful for checking the conditions of beekeeping but not for estimation of geographical origin on the territory of Slovenia, although, this is possible by analyzing different chemical parameters (see previous chapter). Later analysis done on 82 samples of chestnut honey (Kandolf 2011) showed some possibility to identify some specific melissopalynological characteristics related to phytogeographical regions when compared sets of chestnut honey samples between the regions. Although, we don't have a single pollen type that would be always present or at list at specific relative abundance for the specific phytogeographical region, it is possible to compare current sample with existing already analyzed and get the likelihood of the best fit.

Antimicrobial activity of honey and its possible mechanisms in chestnut honey

The main determinants of honey's antimicrobial activity are hydrogen peroxide formation, antimicrobial peptides (AMP), high osmolarity and low pH (Szweda 2017). The antimicrobial activity in all types of honey is significantly influenced by the action of the enzyme glucose oxidase (GOX). It is secreted from the honeybee's feeding glands (hypopharyngeal glands) into the honey. The level of expression of this enzyme in the bee and thus its presence in honey is genetically determined (Bucekova et al. 2014). GOX is an oxidoreductase that catalyzes the oxidation of glucose to gluconic acid during the dilution of honey with water in the presence of oxygen, thereby lowering the pH of honey and producing one molecule of hydrogen peroxide. Both low pH and hydrogen peroxide have antimicrobial activity (Kwakman and Zaat 2012). The general mechanism for inhibiting bacterial growth in honey is its high osmolarity - honey consists of about 80% (w/v) sugar. Other antimicrobial factors are more specific and can vary considerably between different types of honey. For example, an important antimicrobial agent in Manuka honey is methylglyoxal (MGO), which is of plant origin (Mavric et al. 2008; Kwakman and Zaat 2012). By reducing the ability of bacterial adherence and movement, it inhibits both Gram-positive and Gram-negative bacteria. It has been found that polyphenols entering honey from plant nectar greatly increase the antibacterial activity of hydrogen peroxide in honey (Kwakman and Zaat 2012, Bucekova et al. 2018). An example of

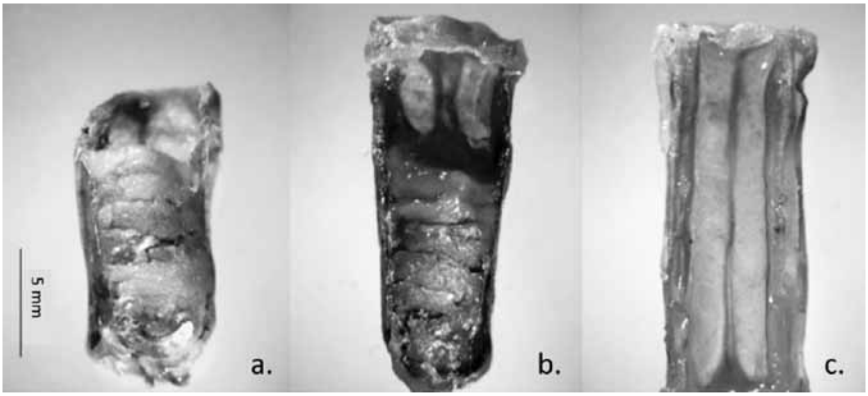

Figure 1: We can find different cell types at the same bee comb with stored food a) comb cell with bee bread, b) comb cell with bee bread covered by honey and closed by wax cap and c) closed comb cell with honey content. In the same comb frame at the same source of nectar, honey above bee bread had significantly higher antimicrobial activity than honey in comb cells without bee bread (Podrižnik and Božič 2015). Photo: B. Podrižnik)

Slika 1: V istem satu lahko najdemo različne tipe satnih celic s skladiščeno hrano, a) satna celica s čebeljim kruhkom, b) satna celica s čebeljim kruhkom pokritim z medom, ki je zaprta z voščenim poklopcem in c) zaprta satna celica, ki vsebuje samo med. V istem satu in pri istem viru medičine ima med, skladiščen nad čebeljim kruhkom, znatno večjo protimikrobno aktivnost, kot med v satnih celicah brez čebeljega kruhka (Podrižnik and Božič 2015) (Slika: B. Podrižnik).

such honey is melon honeydew (Bucekova et al. 2018). Antimicrobial peptides may also be a very important factor in the antimicrobial activity of honey (Kwakman et al. 2010). Their content and type can vary greatly between different types of honey (Bucekova et al. 2018, Erban et al. 2019). Some types of honey, e.g. Manuka and Kanuka, practically do not contain them, while they are abundant in others, e.g. sunflower or linden.

Chestnut honey has the strongest antimicrobial activity among Slovenian honeys, as evidenced by growth inhibition tests of selected bacterial and fungal species (Kunčič et al. 2012). However, it is not yet known what kind of AMP and GOX activity chestnut honey has. Additional antimicrobial activity was observed in chestnut honey stored over bee bread (pollen stored in honeycomb cells, Fig. 1) (Podrižnik and Božič 2015). The additional processing of honey by bees and the effect of stored pollen probably contributed to the fact that under laboratory conditions with the agar diffusion method a 30% larger zone of growth inhibition in *Staphylococcus aureus* was observed. It is difficult to explain which of the factors described above are decisive.

Influence of food processing glands on honey quality

Food processing glands have a key role in final quality of the honey (Winston 1991). Excretion of the gland secretions of the foraging bee starts already during collection of the nectar. Among glands, the subesophageal glands, also known as feeding glands, are the most important and intensively studied contributor to honey processing. Foraging bees secret mainly two enzymes from these glands. The first is alfa–glucosidase, which separates glucose from compound sugars attached by an alfa bound, and it is most effective when splitting sucrose into simple sugars, for this reason it is also called sucrose (Kubo et al. 1996). The second enzyme is glucose oxidase (GOX) (Takenaka et al. 1990), which contributes to antimicrobial activity of the honey by producing hydrogen peroxide through oxidation of glucose into gluconic acid. Peroxidal activity is the major antimicrobial activity in honeys from melliferous plants (see discussion before). Secretion of glucose oxidase is also present in younger nurse bees which are feeding larvae with royal jelly. During this period, nurse bees are secreting proteins of royal jelly, among them antimicrobial peptides (Kubo et al. 1996, Fujita et al. 2010). The dynamic of well-known antimicrobial peptide defenzin-1 secretion is unclear, especially regarding age of the bee and her role in the colony. Young bees are contributing to royal jelly also with the secretions from the mandibular glands. This gland contributes the fatty part of the royal jelly, with 10-hydroxy-decanoic acid the best known (Huo et al. 2016). Some components of the royal jelly also end up in honey due to intensive food exchange between hive and foraging bees (Crailsheim 1991). The involvement of salivary glands in these processes is unclear. It is known that these glands are more involved in other bees in production of pheromones (Feng et al. 2013; Martin et al. 2018).

Excretion of AMP from food processing glands in honey bees

AMP can be an important factor in the antimicrobial activity of a particular type of honey. They are evolutionarily very old defense molecules and represent an important part of the innate immune system. Therefore, they are found in very different organisms as well as in insects. In general, they are amphipathic, cationic peptides, which kill the bacterium by permeabilization of its cell (cytoplasmic) membrane. Several types of AMP are known in bees. While proline-rich apidaecins (Casteels et al. 1993) and abaecin (Casteels et al. 1990), the former 18 to 20 and the latter 34 amino acid residues long, have not yet been detected in honey, defensins, hymenoptaecine and jeleines (Kwakman and Zaat 2012) have all been found.

The most known and researched bee AMP are defensins (Bilikova et al. 2015). Genes for two structural forms of defensin, defensin-1 and defensin-2 (Klaudiny et al. 2005), have been discovered in the genome of the honey bee (*Apis mellifera*). The better understood defensin-1 is constitutively expressed in pharyngeal, hypopharyngeal, and mandibular glands of the bee. Defensin-2, which is expressed in fat and hemolymph, is 55.8% structurally identical to defensin-1 and appears to be expressed, *i.e.* inducible, only in the case of infection. Defensin-1 consists of 51 amino acid residues (Fujiwara et al. 1990) and defensin-2 consists of 43 (Klaudiny et al. 2005), both of them containing 3 disulphide bridges. Bee defensins show a high degree of polymorphism. This can significantly affect the level of their expression and their antimicrobial activity. While defensin-1 is responsible for the collective immunity of bees, defensin-2 is responsible for their individual immunity (Ilyasov et al. 2012). Defensin-1 is a common component of royal jelly (Ramanathan et al. 2018) and honey (Erban et al. 2019). Because it was first discovered in royal jelly, it is also known by an alternative name - royalisin. Defensin-2 has not yet been detected in honey. Defensin-1 is primarily directed against Gram-positive bacteria, but is also toxic to some Gram-negative bacteria (Bilikova et al. 2015). Importantly, it is quite effective in destroying multivariate bacterial biofilms (Sojka et al. 2016). It is interesting to note that although defensin-1 has an antibacterial effect, it also promotes the healing of open wounds. By stimulating the secretion of MMP-9 metalloproteinase from keratinocytes, it stimulates their migration and thus wound closure and re-epithelialisation (Bucekova et al. 2017). Hymenoptaecin (Casteels et al. 1993) is a 93 amino acid residue long AMP, which is inducibly expressed in the case of infection. This is probably the reason why it is only occasionally found in honey (Erban et al. 2019). Its bactericidal activity is broad and directed against both Gram-positive and Gram-negative bacteria. Honey also contains the protein MRJP1 (Major Royal Jelly Protein 1) (Erban et al. 2019). This 61 kDa protein (Tian et al. 2018) is a precursor of three antibacterial peptides, jellein-1, 2, and 3 (Fontana et al. 2004). Jelleins, peptides with a length of 8 to 9 amino acid residues, are probably formed in situ after proteolytic processing of the C-terminal part of MRJP1(Buttstedt et al. 2014) by serine proteases that are also present in honey. The content of MRJP1 was high in all honeys tested so far, so the contribution of jelleins to the antimicrobial activity of honey is probably important. They are toxic to bacteria, both G^{+} and G^{-}, and to yeast (Fontana et al. 2004).

Microorganisms in honey

Honey itself is not a sterile food; it contains both bacteria and fungi (yeasts and molds). However, due to its high osmolarity and low pH, only certain groups of microorganisms can thrive in it, and this community is usually stable. They even found that some bacteria in this community produce bacteriocins that prevent the growth of other bacteria (Szweda 2017). Thus, among the Gram-positive bacteria we can find representatives of the genera *Bacillus, Micrococcus, Streptococcus* and *Clostridium*, while among the Gram-negative bacteria species of the genera *Achromobacter, Citrobacter, Enterobacter, Erwinia, Escherichia, Flavobacterium, Klebsiella, Proteus* and *Pseudomonas* are present. Fungal contaminants are often species of the genera *Penicillium, Aspergillus, Saccharomyces, Zygosaccharomyces* and sometimes representatives of the former genus *Torulopsis*. Secondary sources of microbial contamination in honey may be humans, equipment, utensils, wind, dust, etc. Most bacteria and other microorganisms cannot grow or multiply in honey; they are dormant due to the antibacterial activity of honey. Thus spore-forming microorganisms such as *Bacillus cereus, Clostridium perfringes* and *Clostridium botulinium*, or more precisely their spores, can survive in honey at low temperatures for several months or even a year (Olaitan et al. 2007). The worst possible contamination route is contamination due to poor beekeeping practices, which can be avoided by appropriate beekeeping measures during beekeeping and honey delivery. Disease caused by honey consumption has rarely been suspected, but it has never been directly demonstrated that honey is one of the main sources of infection (Grabowski and Klein 2017). Unfortunately, possible contamination from environmental sources cannot be completely avoided. In such a case, honey can be treated with γ radiation (Jo et al. 2005), and some new research also suggests the use of high pressure and ultrasound (Leyva-Daniel et al. 2017, Janghu et al. 2017). Together with the improvement of honey quality, we must ensure that during the production of the honey product there is no contamination with microorganisms that could cause health problems (Snowdon and Cliver 1996, Olaitan et al. 2007, Silva et al. 2017).

Chemical contamination of chestnut honey and legislation in this field

Environment pollution is reflected also in the contamination of honey. Amounts of pollutants like metals, organic pollutants and biocides rarely exceed regulated minimal residual levels for humans (Al-Waili et al. 2012). Older EU legislation required honey to be completely pollutant-free (Directive 1974). Honey is now included in newer general directive about food products that regulates labeling, residues of pollutants and analytical methods (European Commission 2018). In recent research of Slovene honey samples, Česnik et al. (2019) did not found excessive amounts of pollutants according to current legislation. Nevertheless, remedies of miticides used to fight Varroa mites are often detected, in a smaller extent even in the samples from organic beekeeping (Česnik et al. 2019). French researchers (Wiest et al. 2011) as well Italian (Saitta et al. 2017) also reported on residues of miticides and fungicides. In Italian research (Saitta et al. 2017) low contamination of chestnut honey with pollutants was found. Chestnut nectar contains a lot of minerals (see chapter "Geographical variability…"), so it is not a surprise that chestnut honey was rich with radioactive ^{137}Cs (Panatto et al. 2007). For medical use, honey must be produced under organic standards (Hermanns et al. 2020) and it is necessary to be cautious about all possible sources of contamination, especially about the use of illegal chemicals to treat bee diseases (e.g. acaricides and antibiotics). Even though that bees forage on chestnut in the forest, the chestnut honey could be contaminated with nectar from melliferous plants in intensive agriculture area, as well as the treatments against Varroa mites.

Additional interesting medical properties of honey

In addition to the antimicrobial activity, honey has many other biological activities that are therapeutically interesting, especially in wound healing process (Oryan et al. 2016). Honey has proven antioxidant and anti-inflammatory activity and stimulates immune system cells involved in wound healing and tissue regeneration. Antioxidant activity is driven by a whole range of substances, such as flavonoids, phenolic acids, ascorbic acid, tocopherols, antioxidant enzymes, specific amino acids, and selenium. Among them, phenolic compounds have the greatest antioxidant role, e.g. gallic acid, which together with antimicrobial activity contribute to wound healing. Their role is primarily the reduction of reactive oxygen and nitrogen species and thus the reduction of oxidative reactions in inflammation.

The specificity of chestnut honey is that it has a sufficiently high content of kynurenic acid (Turski et al. 2016). Kynurenic acid is a metabolic product of the amino acid tryptophan. It is also a natural metabolite in human body, where it plays an important role in regulation of metabolism (Milart et al. 2019), immune response (Małaczewska et al. 2014) and neurotransmission. Alteration in homeostasis of kynurenic acid and its metabolites has been implicated in different neurological and psychological disorders (Meier 2019). It is a natural component of human breast milk and regulates weight gain in breast-fed infants. Insufficient levels of kynurenic acid in artificial milk formulas contribute to overweight in infants, which is a risk factor for obesity in later stages of child development (Milart et al. 2019). It has also been proven that kynurenic acid and other tryptophan derivatives are involved in the healing of superficial wounds (Poormasjedi-Meibod et al. 2014, Matysik-Woźniak et al. 2017). High proline content and biochemical conditions in honey also allow the synthesis of additional kynurenic acid derivatives as 3-pyrrolidinyl quinurenic acid and its gamma lactone (Beretta et al. 2009). Their pharmacological activity, as well as the possible role of other specific chemical substances from chestnut honey, is unknown (Truchado et al. 2009a,b,c).

Examples of medical use and opportunity of the chestnut honey

Complications due to the bacterial resistance to classical antibiotics triggered biomedical research on the potential use of honey for wound care (Molan 1992). Researchers tested different types of honey, among them only few were recognized as medical. The first recognized medical honey was monofloral Manuka from New Zealand, and another early example was Revamil® Neatherland, but they both differ in their mode of action (Kwakman et al. 2011). Recently, new products for wound care from different producers have emerged (Hermanns et al. 2020).

Substantial antibacterial and antifungal activity was found in Slovene chestnut honey (Kunčič et al. 2012) and therefore it was recommended for medical use. This knowledge was used in the commercial product line Vivamel® (Fig. 2). We can expect more specific solutions on the market based on specific properties of individual type of honey along with clearer definition of medical honey quality (Hermanns et al. 2020). Chestnut honey has an excellent opportunity for product development, especially because of pharmacological relevant concentrations of kynurenic acid and other derivatives of the kynurenic pathway.

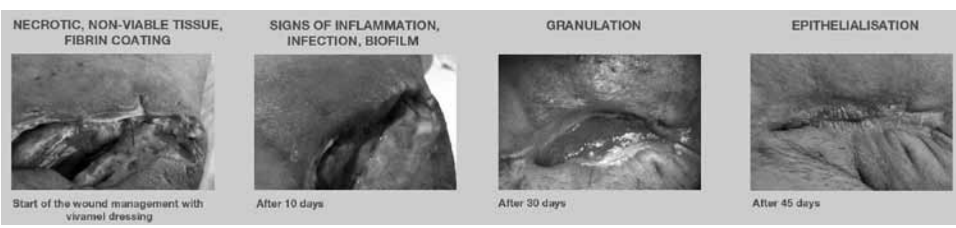

Figure 2: Demonstration of wound healing with use of chestnut honey. (Figure is from directions for use of wound dressing with medical honey by Tosama d.o.o.)

Slika 2: Prikaz procesa celjenja ran z uporabo kostanjevega medu. (Slika je izrezana iz “Smernice za uporabo oblog z medicinskim kostanjevim medom”, Tosama d.o.o.)

Conclusions

1. Slovene chestnut honey is relatively well characterized from all quality expects. Differences in its physical and chemical characteristics are due to its geographical origin.
2. Melissopanology is a good tool to verify beekeeping practice regarding specific monofloral production of the chestnut honey, also partially for geographical origin.
3. The antimicrobial activity of chestnut honey is considerable but also varies and requires further study. Antimicrobial peptides probably contribute strongly to the overall antimicrobial activity of the chestnut honey.
4. Activity of the hypopharyngeal glands during honey processing is the major source of enzymes and antimicrobial peptides in the honey.
5. The production of honey for medical purposes requires special attention to avoid microbial contamination. Organic beekeeping is the safest way for medicinal honey production, but even in this case, one must be aware of potential contamination by pollutants.
6. Chestnut honey has unique medical properties with pharmacologically relevant concentrations of kynurenic acid and other compounds derived from the kynurenic metabolic pathway.
7. Chestnut honey is well recognized in the field of wound care. New findings may lead to novel specific applications as medicinal honey.

Povzetek

Kostanjev med je bil od nekdaj prepoznan kot nekaj posebnega, predvsem v evropskem prostoru. Tudi laiki lahko hitro prepoznajo nekaj ključnih lastnosti kostanjevega medu – specifično temno jantarno barvo in grenek okus. Za to vrsto medu je značilna dolgo obstojna aroma (Bertoncelj s sod. 2011a), zato je senzorična sprejemljivost te vrste medu, predvsem med mlajšimi potrošniki, slabša. V zadnjih desetletjih se tudi za med pravega kostanja kopičijo znanstveni podatki o različnih lastnostih, predvsem kemijski sestavi medu, pridružil pa se je tudi naboru vrst medu, ki so bile prepoznane kot primerne za medicinsko uporabo. Značilno za kostanjev med je visoko razmerje med fruktozo in glukozo, ki znaša okoli 1,5, zato tipičen kostanjev med običajno ne kristalizira (Tab. 1). V primerjavi z ostalimi vrstami slovenskega medu je za kostanjev med značilna visoka električna prevodnost, visoka vrednost pH, velika vsebnost aminokisline prolin in visoka aktivnost encima diastaze (Tab. 2). Kostanjev med v primerjavi z ostalimi vrstami slovenskega medu (akacijevim, lipovim, hojevim, smrekovim, cvetličnim in gozdnim medom) vsebuje največ K^+, Ca^{2+} in Mn^{2+} ionov (Kropf s sod. 2010). Kostanjev med je tudi relativno bogat s fenolnimi spojinami (Bertoncelj s sod. 2011b), kar se odraža tudi o relativno visokih antioksidativnih učinkih, primerljivo z antioksidativno učinkovitostjo maninih medov (Korošec s sod. 2016b). Kropf in sod. (2010) so analizirali sestavo medu iz različnih geografskih regij Slovenije (Perko 1998) in vrednotili različne parametre. Kostanjev med iz panonske makroregije je vseboval več rubidija, med iz dinarske makroregije pa je bil statistično značilno temnejše barve in je vseboval manj prolina. Z metodo linearne diskriminantne analize (LDA) so dokazali, da ima najpomembnejši prispevek k razlikovanju slovenskega kostanjevega medu glede na geografski izvor vsebnost žvepla, pepela in kalija, parameter barve L^* (svetlost) ter razmerje S/Ca (Kropf s sod. 2010). Fenološko v več kot polovici vzorcev kostanjevega medu najdemo poleg močno prevladujočega pravega kostanja tipe cvetnih prahov kot so *Trifolium repens*, *Tilia* sp. Plantago sp. *Asteracea* tip J in *Filipendula* sp., ki cvetijo sočasno s pravim kostanjem (Golob s sod. 2008, Kandolf 2011). V več kot polovici vzorcev so bili prisotni tudi tipi cvetnih prahov sadnega drevja in javorjev (*Acer* sp.), ki cvetijo pomladi pred pravim kostanjem, kar nakazuje na težavo pridelave čistega monoflornega medu, lahko pa tudi zaradi primesi zaradi skladiščenja kostanjevega medu nad čebeljim kruhkom starejšega izvora.

Protimikrobna aktivnost pri vseh vrstah medu je pomembno pogojena z delovanjem encima glukoza oksidaze (GOX) in z njim povezanim vodikovim peroksidom (Szweda 2017). GOX se v med izloča iz čebeljih krmilnih žlez, poleg encimov pa te žleze izločajo tudi protimikrobne peptide (AMP) (Bucekova s sod. 2014). Polifenoli, ki v med pridejo iz rastlinskega nektarja, močno ojačajo protibakterijsko delovanje vodikovega peroksida v medu (Kwakman and Zaat 2012, Bucekova s sod. 2018). Ostali protimikrobni dejavniki so bolj specifični in se lahko med različnimi vrstami medu precej razlikujejo. Tako je ključni protimikrobni dejavnik medu manuka metilglioksal (MGO), ki je rastlinskega izvora (Mavric s sod. 2008, Kwakman in Zaat 2012). Kostanjev med ima od slovenskih vrst medov najmočnejšo protimikrobno aktivnost, kot je bilo dokazano s testi inhibicije rasti izbranih bakterijskih in glivnih vrst (Kunčič s sod. 2012). Vendar pa še ni poznano, kakšne vsebnosti AMP in aktivnosti GOX ima kostanjev med. Dodatna protimikrobna aktivnost je bila opažena v kostanjevem medu, skladiščenim nad čebeljim kruhkom (cvetni prah, skladiščen v satnih celicah, sl. 1) (Podrižnik in Božič 2015). Encime, AMP in še druge sestavine se izločajo v med iz čeljustnih žlez čebel (Winston 1991), vanj pa zaidejo tako med nabiranjem kot predelavo v panju (Crailsheim 1991). Najbolj znani in raziskani čebelji AMP so defenzini (Bilikova s sod. 2015), med njimi se defenzin-1 izraža v podžrelni žlezi, ki poleg proti mikrobnega delovanja pospešuje tudi celjenje odprtih ran. V medu se nahaja tudi protein MRJP1 (od angl. »Major Royal Jelly Protein 1«) (Erban s sod. 2019). Ta 61 kDa protein je prekurzor treh protibakterijskih peptidov, jeleina-1, 2 in 3, ki so toksični za bakterije, tako G^+ kot G^-, kot tudi za kvasovke (Fontana s sod. 2004).

Sam med ni sterilno živilo, v njem najdemo tako bakterije kot glive (kvasovke in plesni). Vendar lahko zaradi visoke osmolarnosti in nizkega pH uspevajo le določene skupine mikroorganizmov, ta združba je večinoma stabilna. Ugotovili so celo, da nekatere bakterije v tej združbi producirajo bakteriocine, ki preprečujejo razrast drugih bakterij (Szweda 2017). Sekundarni viri mikrobne kontaminacije v medu so lahko ljudje, oprema, posoda, veter, prah itd. Na žalost se možni kontaminaciji iz okoljskih virov ne moremo popolnoma izogniti. V takšnem primeru lahko med obdelamo z γ žarčenjem (Jo s sod. 2005), nekatere nove raziskave pa predlagajo tudi uporabo visokega pritiska in ultrazvoka (Leyva-Daniel s sod. 2017, Janghu s sod. 2017). Onesnaženje okolja se odraža tudi v onesnaženosti medu. Vrednosti onesnažil, kot so kovine, organska onesnažila in biocidi redko presežejo predpisane mejne vrednosti za človeka (Al-Waili s sod. 2012, European Commission 2018, Česnik s sod. 2019). Za medicinske namene naj bi se primarno uporabljal med pridobljen po ekoloških standardih (Hermanns s sod. 2020), vseeno pa je potrebno biti pozoren na vse možne vire onesnaženja, zlasti morebitno zlorabo nedovoljenih sredstev za zatiranje bolezni (n.p. akaracidi in antibiotiki).

Posebnost kostanjevega medu je, da vsebuje kinurensko kislino (Turski s sod. 2016). Kinurenska kislina je presnovni produkt aminokisline triptofan. Je tudi naravni metabolit v človeškem telesu, kjer ima pomembno vlogo pri uravnavanju metabolizma (Milart s sod. 2019), imunskega odziva (Małaczewska s sod. 2014) in prenašanju živčnih signalov. Dokazano pa je tudi, da so kinurenska kislina in drugi derivati triptofana udeleženi v procesih celjenja površinskih ran (Poormasjedi-Meibod s sod. 2014, Matysik-Woźniak s sod. 2017). Zapleti pri zdravljenju ran zaradi odpornosti bakterij na klasične antibiotike je stimulirala biomedicinske raziskave potencialne uporabe medu za pripravke za nego ran (Molan 1992). Znatno protibakterijsko in protiglivično delovanje je bilo potrjeno za slovenski kostanjev med (Kunčič s sod. 2012) (Sl. 2). Z jasnejšo opredelitvijo kvalitete za deklaracijo medicinskega medu (Hermanns s sod. 2020) bodo na trgu ponujene nove rešitve na osnovi specifičnih lastnosti posameznih vrst medu. Tu ima pravi kostanj posebno priložnost, predvsem zaradi farmakološko relevantnih koncentracij kinurenske kisline in drugih derivatov kinurenske poti.

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