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DETERMINATION OF SOME QUALITY PARAMETERS OF HONEY BEE FEED

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Abstract: Due to an increasing deficiency in feed for bees, beekeepers increasingly tend to use commercial industrial sugar (sucrose) in the nutrition of bee colonies. In the bees nutrition, sugar can be used as liquid sugar - syrup (diluted in water in different ratios). In addition, sugar can be used to make sugar dough (candy paste). Beekeepers often add enzymes or acids to invert the sugars and speed up the process with additional heating. When the bees are fed syrup that has not been overheated no serious problems arise. But in a case when overheated or multiple heated syrups and the syrup hydrolyzed/inverted by inorganic acids are used in the nutrition the occurrence of hydroxymethylfurfural (5-hydroxymethyl-2-furancarboxaldehyde-HMF) which can shorten a life span or cause bee mortality. The purpose of this study is to determine concentrations of HMF, reducing sugars, sucrose, free acids and pH value of solid and liquid feed for bees in the samples of candy paste and syrup, with a particular focus on HMF. During the 2012-2021 period in the laboratory of the Veterinary Specialized Institute "Kraljevo" (VSI "Kraljevo"), in Kraljevo 42 samples of candy paste, 23 samples of enzyme hydrolyzed sugar (syrup) and 6 samples of acid and/or temperature inverted or diluted sugar (syrup) were analyzed. Out of 68 food samples analyzed for the presence of HMF 8 samples (11.76%) were positive for the presence of HMF in concentrations higher than 40 mg/kg.

Key words: feed for bees, hydroxymethylfurfural, sugars, free acidity, pH

Introduction

Owing to increasing climate changes, intensive farm management, single-crop farming, deforestation, and large-scale melioration a considerable part of an area of bee pasture is being reduced (Quinlan et al., 2023). Taking into consideration a lack of food sources, beekeepers more and more turn to various alternatives wishing to replace nectar and pollen by artificial food. When you type "honey bees feeding" into your browser you receive lots of articles and research papers whose number has, over the last 20 years, increased from about 100 to over 700 annually (Graph 1). Therefore the growing interest both of beekeepers from the region and some firms to produce suitable food for bees that will in certain periods of the year replace nectar in bee nutrition is not surprising.

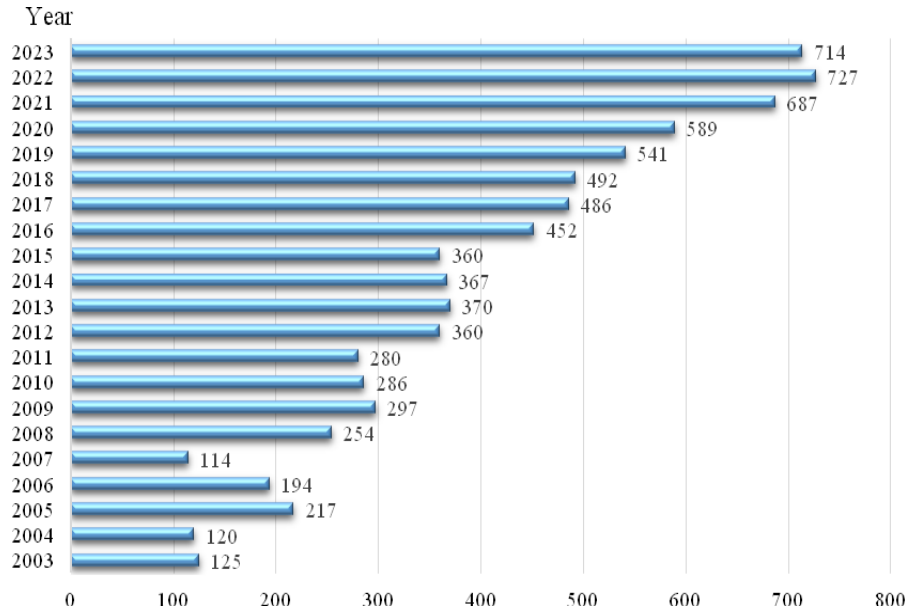

Figure 1. Number of published articles in period of last 20 years (this data was taken from the Science Direct database using one research query "Honey bees feeding")

Qualitatively and quantitively balanced nutrition plays a primary role in maintaining healthy and strong bee colonies. To satisfy their needs the bees collect nectar, honeydew, pollen, resinous substances and water. Nectar provides them with carbohydrates to satisfy their energy needs and pollen provides them with proteins for growth and development. Other nutritive components obtained mostly from nectar and pollen (lipids, minerals, vitamins, enzymes, colored and odorous

substances, etc.) supplement their nutrition (Standifer et al., 1977). However, in certain months, years and/or in some regions the food for bees can be scarce or even absolutely lacking. Hence, beekeepers have to provide additional sources of carbohydrates to feed their bee colonies (Haydak, 1970; Krainer et al., 2016; Paray et al., 2021; Tsuruda et al., 2021). In bee nutrition sugar is the most often used (obtained from sugar beet or sugar cane) in the form of liquid food. It is diluted with water in different ratios and used in the form of liquid food. Besides this, high fructose corn syrup or starch syrup are used as well (Brodschneider et al., 2010; Krainer et al., 2016). A solid carbohydrate food – sugar dough (candy paste) can be made out of sugars. Jevtić et al. (2003) reported that for the development of colonies in spring, the addition of one candy paste in 3 different terms in the preceding winter period had the most favorable effect. The addition of two candy pastes in three terms harmed spring development and resulted in the death of colonies.

The beekeepers often add enzymes or acids for sugar hydrolysis/inversion and accelerate a whole process by extra heating. The additional carbohydrated food increases the strength of colony, prevents starvation and decreases excessive winter losses (Emsen and Dodologlu, 2014). In our climate conditions additional nutrition is necessary in late summer and in early autumn when bees make their winter supplies but also in the spring when the colonies are stimulated to produce a large amount of brood. Beekeepers often produce syrup themselves by mixing sugar and water and by adding some acidifying agents such as acetic and lactic acids (Standifer et al., 1977).

If the bees are fed a syrup that has not been overheated and that has been supplemented with enzymes (*invertase*) for hydrolysis there are no big problems with bee products or with bee health. The additional feeding with sugar syrup or solid food, particularly with that of a weak quality, can lead to several problems. The first is the adulteration of honey and the second is that harmful substances found in syrup or candy pastes (5-hydroxymethyl-2-furancarboxaldehyde/hydroxymethylfurfural/HMF, acids) can get into the honey (Kanelis et al., 2022; Schrenk et al., 2022). Adulteration of honey can occur by feeding the bees with sucrose syrup which is made from sugar beet or by adding industrial sugar syrups obtained from starch: by a thermal, acid or enzyme inversion (Ruiz-Matute et al., 2007). An excessive use of these feeds in the time of bee major pasture can get into a final product honey and is considered as adulterated honey (Bogdanov, 2006; Guler et al., 2007; Guler et al., 2014; Dodd et al., 2024). If in bee nutrition we use an overheated or multi-heated sugar syrup or the one hydrolysed by acids (most often inorganic) in which there occurred a formation of HMF, a life span of a worker bee can be shortened while higher consumption may even be lethal (Schrenk et al., 2022). HMF is a cycling aldehyde that is most often created by dehydration of fructose and glucose in an acid environment. At first, the presence of HMF was an indicator of adulteration of

honey but nowadays it is primarily the indicator of heating (decrystallisation) and freshness of honey. The concentration of HMF in honey increases by shelf life and besides temperature and time of storing it depends also on the type of honey, its pH, share of acids and humidity. A high quantity of HMF (more than 100 mg/100g honey) in the first 15 days of feeding does not negatively affect the worker bees. However, further feeding by such food (15-30 days) causes an abrupt dying of the cells of midgut and increases the mortality of such fed bees (Gregorc et al., 2020). After 30 days of feeding only a control treatment had more than 20% alive bees while other treatments (100-1500mg/100kg) had below 10% survived individuals. Somewhat higher mortality of bees fed by syrup containing 150 mg/kg HMF was reported by LeBlanc et al. (2009), who said that 50% adult individuals died after 19 days of feeding by such syrup. Ceksteryte and Racys (2006) think that bees can metabolize a low quantities of HMF in stored food. Of the similar opinion are also Jachimowicz and El Sherbiny (1975) who think that concentration of 30-48 mg/kg HMF ought not be harmful for worker bees.

Numerous studies have shown that HMF can be toxic for adult bees (Schrenk et al., 2022). Bailey (1966) determined that honey which has been stored for a long time contains an increased quantity of HMF, causing increased bee mortality in contrast to fresh honey. Jachimowicz and El Sherbiny (1975) determined that concentration of HMF of 30 ppm did not cause a significant mortality in adult individuals. In addition, they determined that a high quantity of HMF (150 ppm) in syrup which was produced by acid hydrolysis significantly increases bee mortality in bees fed by this syrup. Taking this into consideration, Krainer et al. (2016) studied the impact of HMF on mortality of larvae, pupa and juvenile bees of 2-22 days old. They confirmed that concentration of HMF of 5-750 ppm does not affect increased mortality but that concentration of 7500 ppm leads to complete dying of larvae and pupae.

An even bigger problem with HMF can arise during preparation of a solid feed. During the preparation of syrup for candy pastes it is often overheated or inverted by inadequate substances (inorganic acids), therefore in this way a great quantity of HMF can be generated in feed.

Smodiš Škerl and Gregorc (2014) fed 50 bees in cages with candy pastes from commercial production. The concentration of HMF in candy pastes was less than 10 mg/kg HMF in the first two candy pastes, 437 in the third and in the fourth 914.6 mg/kg HMF. Bees fed by candy pastes with lower concentration of HMF lived significantly ($p < 0.0001$) longer (up to 27 days) compared with the bees fed candy pastes with higher HMF (20-24 days).

Materials and Methods

In the period from 2012-2021 in the laboratory of the Veterinary Specialised Institute "Kraljevo" (VSI "Kraljevo"), in Kraljevo, the samples of candy paste by enzyme hydrolysed (inverted) sugar and acids and/or by temperature inverted or diluted sugar were analysed in order to study 5 parameters of quality of food for bees: HMF, reducing sugars, sucrose, free acids and pH (Table 1). The parameters were analysed in 42 samples of sugar dough, 23 samples of enzyme hydrolysed sugar (syrup) and 6 samples of acidifying and/or by temperature inverted or diluted sugar (syrup) in laboratory conditions. The samples were delivered by beekeepers and firms from 13 municipalities of central and southwest Serbia. Since neither in the Republic of Serbia nor in the world, there is a legal framework which regulates the way of production, parameters of quality and declaration for bee feed (Paray et al., 2021; Tsuruda et al., 2021), each sample was analysed for one or more different parameters of quality at the personal request of each customer.

Table 1. Type of feed for bees, number of laboratory analysis done per types of food and number of processed parameters of quality

Food	HMF	Reducing sugar	Sucrose	Free acids	pH
Candy paste	42	17	16	5	1
Enzyme inverted sugar (syrup)	20	23	22	3	12
By acids and/or by temperature inverted or diluted sugar (syrup)	6	6	6	-	3
Σ	68	46	44	8	16

The quantitative method for the determination of HMF, reducing sugars (%) and sucrose content (%) were analyzed following the International Honey Commission (Bogdanov et al., 2002).

The free acidity was determined by the titrimetric method, using NaOH and HCl. Ten grams of each sample was dissolved in 75 ml distilled water in 250 ml beaker. It was stirred with the magnetic stirrer, the pH electrodes immersed in the solution and pH recorded. It was titrated with 0.1M NaOH to pH 8.30 (a steady reading should be obtained within 120 sec of starting the titration; in other words, complete the titration within 2 minutes). Recorded the read to the nearest 0.2 ml when used a 10 ml burette and to 0.01 ml if the automatic titrator has sufficient precision. Results were expressed as mEq/kg.

The values of pH were read on the pH meter Scholar 425 (Corning, USA).

Results and Discussion

Neither in the Republic of Serbia nor in the world there is a legislation on the quality of feed for bees (Paray et al., 2021; Tsuruda et al., 2021). There are some legal frameworks regarding the quality and health safety of honey and some bee products. If it is known that honey is the best food for honey bee then the other food as well should meet the standards set for honey. It must be admitted that all the parameters of honey quality cannot be applied to the food for bees as well because some feeds (sugar dough) are produced mainly from sucrose. Thus the application of the Rule book on Quality of Honey and Other Bee Products (2015) can refer only to some of the parameters: HMF, reducing sugars and free acids.

Out of total of 68 samples analysed in the laboratory for the presence of HMF the highest level of HMF was determined in the samples of candy paste for bees (Table 2).

Table 2. HMF concentration in feed for bees (mg/kg)

	Number of samples	Mean ± SD	Number of samples >40 mg/kg	Min	Max	Coefficient of variation (%)
Candy paste	42	31.18±52.62	6	0.3	212.16	168.76
Enzyme inverted sugar (syrup)	20	17.40±33.23	2	0.3	127.49	190.99
By acids and/or by temperature inverted or diluted sugar (syrup)	6	2.6±0.87	0	1.67	3.5	33.96
Σ	68		8			

The highest average concentration of HMF was determined in candy paste and the largest number of samples (6) contained more than 40 mg/kg HMF. By a laboratory analysis of enzymatically inverted sugar yielded slightly better results, as it had 55.6% less HMF compared to candy paste. Only two out of twenty laboratory processed samples had more than 40 mg/kg HMF. In the samples of acidifying and/or by temperature inverted or diluted sugar an average concentration of HMF was 2.6 mg/kg, while none of the samples had a concentration higher than 40 mg/kg. It should be taken into consideration that the smallest number of samples of this feed was analysed but it is obvious that the feed was prepared in a suitable way meaning there was neither overheating nor repeated heating.

If sucrose is used for inverting the syrup and if there is no overheating and/or repeated heating HMF does not exceed the allowed limits. In all the samples

of syrup obtained at different temperature values (35-65 ºC), the concentration of HMF was lower than 4.32 mg/kg (Radovanović et al., 2017).

A concentration of reducing sugars indicates the level of hydrolysis of sucrose in table sugar rendered into glucose and fructose. This parameter was determined in a total of 46 food bee samples (Table 3).

Table 3. Reducing sugars in feed for bees (%)

	Number of samples	Mean±SD	Number of samples <60%	Min	Max	Coefficient of variation (%)
Candy paste	17	17.88±9.34	17	7.76	40.0	52.24
Enzyme inverted sugar (syrup)	23	54.10±11.28	15	29.12	69.54	20.84
By acids and/or by temperature inverted or diluted sugar (syrup)	6	50.57±19.05	3	13.17	63.32	37.67
Σ	46		35			

All analysed samples of candy paste (17) had an increased content of sucrose, what was expected. A candy paste is made by mixing a ground table sugar and inverted syrup so that sucrose is prevalent in it but there was also a candy paste that contained up to 40% reducing sugars. An average value of the quantity of reducing sugars in syrup obtained by enzyme hydrolysis of sugar shows that in the majority of syrups prepared in this way, the value is close to the Rulebook on Quality of Honey and Other Bee Products (2015). According to this rulebook, a flower honey should not have less than 60 g/100g honey while forest honey should not contain less than 45g/100g. The same values were obtained by acid and/or by temperature inverted or diluted sugar where only one sample had a reduced content of reducing sugars and the others were close to or above the values defined in the Rulebook on Quality of Honey and Other Bee Products (2015). In the research conducted by Frizzera et al. (2020) it was determined that bees fed syrup made from water and sucrose lived longer than bees fed a mixture of water, glucose and fructose (1:1:1).

The honey bee hydrolyses sucrose to reducing sugars by enzyme invertase for which process they spends energy and proteins (Frizzer et al., 2020). The wish of beekeepers to help the bee in this activity seems to be justifiable but not always successful and sometimes even unneccessary (Frizzer et al., 2020). The concentration of sucrose is highest in candy paste while it is considerably lower in enzyme and acid and/or temperature inverted or diluted sugar (Table 4).

Table 4. Sucrose in feed for bees (%)

	Number of samples	Mean±SD	Number of samples >10%	Min	Max	Coefficient of variation (%)
Candy paste	16	73.54±6.5	16	65.06	85.99	8.84
Enzyme inverted sugar (syrup)	22	10.44±9.16	7	2.35	38.4	87.74
By acids and/or by temperature inverted or diluted sugar (syrup)	6	11.85±13.43	1	3.18	38.98	113.3
Σ	44		24			

In the samples of enzyme invert sugars the values of sucrose were increased in several samples, in seven samples the value was higher than 10% while in other samples the values were close to those prescribed by the Rulebook on Quality of Honey and Other Bee Products (2015). Only in one sample of acidified and/or by temperature inverted or diluted sugar the value of sucrose was higher than 10%.

Kanelis et al. (2022) searching for methods to determine adulterated honey proved that feeding the bees by different kinds of food can lead to honey free of sucrose when the bees are fed invert syrup. Somewhat more sucrose was found in the honey produced by bees fed candy paste (3.2%). The highest value of sucrose was determined in the honey made by bees fed sugar syrup (6.2%).

The presence of free acids was determined in 8 samples of food for bees (Table 5).

Table 5. Free acids in feed for bees (mEq/kg)

	Number of samples	Mean±SD	Number of samples >50% mEq/kg	Min	Max	Coefficient of variation (%)
Candy paste	5	11.34±12.96	-	1.6	34.1	114.28
Enzyme inverted sugar (syrup)	3	11.38±15.7	-	1.7	29.5	137.92
By acids and/or by temperature inverted or diluted sugar (syrup)	-					
Σ	8		-			

The values of the abovementioned parameter in the samples of candy paste and invert syrup were below the maximum values permitted by the Rulebook on Quality of Honey and Other Bee Products (2015). The increased acidity in food for bees can arise either by acidification or fermentation of food (alcoholic and vinegar fermentation). During this period, in the samples analysed in a laboratory, regarding the production of food for bees no such issue occurred so that the results were satisfying as well. During the production of food for bees, particularly during inversion, a special attention should be paid to the pH value. An acidification of food for bees by citric or hydrochloric (HCl) acid to pH 2.8 significantly decreases surviving of worker bees in relation to bees fed by acid-free syrup (Frizzera et al., 2020).

When they consume food honey bees reduce its pH value to about 4. This also pertains to royal jelly whose pH value at the moment of secreting is about 7 (Muresan and Buttstedt, 2019). Before it is given as food to larvae royal jelly is mixed with fatty acids produced in maternal glands by which process the pH is reduced to 4-4.5. A majority of the total number (16) of samples analysed on this parameter had the appropriate values (Table 6).

Table 6. pH in feed for bees

	Number of samples	**Mean±SD**	**Min**	**Max**	**Coefficient of variation (%)**
Candy paste	1	4.73			
Enzyme inverted sugar (syrup)	12	4.79±1.29	3.07	6.7	26.95
By acids and/or by temperature inverted or diluted sugar (syrup)	3	4.41±0.87	3.41	4.92	19.71
Σ	16				

By inverting acidic food (pH 2) a large quantity of HMF is being generated, hence, almost only 10 minutes after boiling point the concentration of HMF reaches 1786.7 mg/l. After 40 minutes of boiling the level of HMF increases to 14366.7 mg/l what is very harmful for bees. At higher values (pH 3 and 4) such high quantities of HMF (Frizzera et al., 2020) were not observed.

Conclusions

Upon the laboratory analysis of samples of feed for bees in the laboratory of the Veterinary Specialised Institute "Kraljevo" there are several conclusions to be drawn:

The greatest interest both of beekeepers and firms engaged in the production of feed for bees was shown for the value of HMF. It is obvious by the number of requests for this kind of analysis, 68 samples/analyses in total. Somewhat less interest was shown for reducing sugars and sucrose, while the least interest was shown for the presence of free acids and the pH value.

In eight samples of feed for bees (6 samples of candy paste and 2 samples of acidifying and/or by temperature inverted or diluted sugar) determined value of HMF was higher than 40 mg/kg HMF what can cause problems in bees feeding (weakening and/or dying of bees).

The quantity of reducing sugars in feed produced for bees is significantly lower than those prescribed for honey. Out of 46 samples analysed in 8 samples of enzyme invert sugar and in 3 samples of acidifying and/or temperature inverted or diluted sugar, their value was higher than 60%.

High CV values show that in practice there are no standard methods of food preparation that are used in feeding bees.

The concentration of free acids and pH value of feed for bees were within the values permitted by Rulebook on Quality of Honey and Other Bee Products (2015).

Utvrđivanje određenih parametara kvaliteta u hrani za pčele

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Rezime

Zbog sve većeg nedostatka hrane za pčele, pčelari sve više pribegavaju upotrebi konzumnog šećera (saharoze) u ishrani pčelinjih zajednica. U ishrani pčela šećer se može koristiti kao tečna hrana, sirup (rastvara se vodom u različitim odnosima). Takođe se od šećera može praviti šećerno testo (pogača). Želeći da ubrza proces razgradnje saharoze, pčelari često u vodeni rastvor šećera dodaju sredstva za invertovanje (enzime ili kiseline), a ceo proces ubrzavaju dodatnim zagrevanjem. Ukoliko se pčele hrane sirupom koji nije pregrevan i kome su za invertovanje dodati enzimi (*invertase*) ne javljaju se veći problemi. Ukoliko se u prihrani koristi pregrevan ili više puta zagrevan sirup i onaj koji je hidrolizovan/invertovan kiselinama (najčešće neorganskim) može doći do stvaranja hydroxymethylfurfural-a (5-hydroxymethyl-2-furancarboxaldehyde-HMF) koji skraćuje životni vek pčela

radilica, a pri višim koncentracijama dovodi do njihovog uginuća. Cilj rada bio je utvrđivanje fizičko-hemijskih parametra, čvrste i tečne hrane za pčele u uzorcima šećernog testa i sirupa, prvenstveno HMF-a. U periodu od 2012-2021. godine u laboratoriji Veterinarskog Specijalističkog Instituta "Kraljevo" (VSI "Kraljevo"), u Kraljevu laboratorijski je ispitivan sadržaj HMF, redukujućih šećera, saharoze, slobodnih kiselina i pH hrane za pčele. Ispitano je 42 uzorka šećernog testa, 23 uzoraka enzimski hidrolizovanog šećera (sirupa) i 6 uzoraka kiselinama i/ili temperaturom invertovanog ili samo rastvorenog šećera (sirupa). Od ukupno 68 uzorka hrane za pčele analiziranih na prisustvo HMF-a, u 8 uzoraka (11,76%) utvrđeno je prisustvo HMF-a u koncentraciji višoj od 40 mg/kg.

Ključne reči: hrana za pčele, hodroksi metil furfural, šećeri, slobodna kiselost, pH

Author Contributions
K.M., N.N., J.Ć. and G.J. conceived of the study, and participated in its design and coordination and helped to draft the manuscript. V.K., R.M. and M.D. carried out the lab work. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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