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Research Article

Sensory and antioxidant properties of mead with added beehive products

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Abstract

The aim of this study was to explore the pH, HMF, antioxidant activity, sensory properties and color of four meads with added beehive products (C-control; MPP – with added 1% extract of propolis; MPO – with addition of 1% pollen and MBB with addition of 1% bee bread). Compared to C, the use of propolis, pollen and bee bread significantly decrease pH of the meads. Control mead was light in color. HMF values were below 40 mg.kg^{-1} in all tested samples according to Bulgarian legislation. DPPH values in MBB and MPO was 1.8 and 2 times higher compared to C. ABTS increase 1.17 and 1.32 times after 1% bee bread and pollen addition. MPP mead had highest values of antioxidant activity (DPPH, ABTS, FRAP, CUPRAC) TPC, phenolic acids and flavonoid content. The ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) decrease in the following order: MPP > MPO > MBB > C. According to intensity of smell, balance and acidity of taste and overall experience the higher scores were awarded to MBB and MPO meads. The lowest sensory scores for taste and smell were awarded to MPP mead due to strong intrusive taste of propolis identical to medicine.

Keywords

Beehive products, belvedere, mead, functional properties

Abbreviations

ABTS – 2,2 Azinobis-(3-ethylbenzothiazoline-6-sulfonate); CUPRAC – cupric reducing antioxidant capacity; DPPH – free radical scavenging assay; FRAP – ferric reducing ability of plasma phenolic compounds; HMF – hydroxymethyl-2-furfural; TPC – total phenolic compound

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Introduction

Mead is one of the oldest alcoholic beverages obtained after fermentation of honey. Different meads exist based on the type of honey, the addition of spices and/or fruits, the type of used yeast, and the technological variant of preparation, maturation and aging. Traditional mead is made from honey, water and yeast. Melomel is mead prepared with addition of fruits. If herbs or spices were set the mead is called *metheglin* and *braggot*, is mead made with barley or wheat malt (Hall 1996; Ramalhosa et al. 2011).

The chemical composition of mead is once affected by the origin of the honey and the yeast strains used for fermentation. The added ingredients such as herbs, spices and/or fruits on the other hand modify the sensory characteristics of the mead. The content of biological active components increases with potential health effects on the consumer health. The use and action of added herbs, spices and fruits on quality of meads have been published recently (Angotti 2021; de Oliveira et al. 2021; Freitas et al. 2022; Simão et al. 2023).

In the recent years the consumers pay attention to bee products as propolis, pollen and bee bread, due to growing interest in the physical and mental well-being. Propolis is a natural product composed by resinous and balsamic material and is responsible for the protection of bees and hives (Kumar et al. 2021). Raw propolis contains 40-50% resin, 10–30% wax, up to 6% essential compounds, up to 5% pollen and up to 20% polyphenols. Phenolic acids and flavonoids give strong antioxidant and antimicrobial potential to propolis (Kolayli & Keskin 2020).

Bees collect and pack pollen from flowers into granules. Pollen is a source of proteins, lipids, sterols, vitamins (pro vitamin A, vitamin E, niacin, thiamine, biotin), minerals (Ca, Mg, Fe, Zn, and Cu), carbohydrates, folic acid, co-enzymes, fatty acids, phospholipids, phytosterols, terpenes, carotenoids pigments (i.e., lycopene, and zeaxanthin), polyphenols, phenolic acids, flavonoids, anthocyanins (Kolayli & Keskin 2020). Studies have shown that bee pollen exhibits wide range of bioactive properties, such as antioxidant, anti-inflammatory, anticarcinogenic, antibacterial and anti-neurodegenerative activities.

Bee bread (perga or ambrosia) is stored larva feed, produced from pollen by lactic acid fermentation. Bee bread is highly valuable natural bee product contain quercetin, kaempferol, myricetin, isorhamnetin and herbacetin derivatives with antioxidant and antitumoral activities (Weis et al. 2022).

While a number of studies about mead with added herbs (Freitas et al. 2022), spices (Angotti 2021; de Oliveira et al. 2021) and fruits (Kawa-Rygielska et al. 2021) exist, there is not enough information about mead supplemented with bee products, nor is it officially listed as a mead subspace.

Therefore, the aim of the present study is to investigate the antioxidant activity, sensory characteristics and biologically active components of three types of mead produced with addition of propolis, pollen or bee bread.

Materials and Methods

Honey. Dark polyfloral honey purchased from a local beekeeper from the South-West region of Bulgaria was used to produce the mead. The characteristics and quality of the honey were established in accordance with the requirements of the Bulgarian legislation BSS 3050-80, Ordinance No. 48 of November 11, 2003 on the order and methods of sampling and the methods used for honey analysis. Ordinance on the requirements for bee honey intended for human consumption, adopted by PMS No. 196 of 28.VIII.2002 (SG, No. 85 of 2002).

Yeast strain. Pure culture yeast strain M05 (*Saccharomyces cerevisiae*) from "Mangrove Jack's" company was used for the study, which was suitable for the production of all types of mead and was perhaps the most liked strain and the most frequently used. The strain characterized with high attenuation - 95 - 100%, with an alcohol tolerance up to 18% v/v. They are ester-forming and the esters are mostly floral. Fermentation temperature range – 15 - 30°C.

Mead wort production and alcoholic fermentation. The mead wort production was carried out entirely in a brewing installation for the production of beer wort from the German company "Spidel" with a volume of 25 L. The used ratio was 1:4 – 16 L of water was added to 4 kg of polyfloral

honey. The water was boiled at 100°C for 10 min in the brewing installation. The water was then cooled to 50°C by a coil through which cold water passed and honey was added. The brewing installation pump was used to recirculate the wort and mix it well so that it was homogenous. Then the water-honey mixture was cooled to 25°C. The mead original extract was 15% w/w, which was determined dosimetrically with Anton Paar DMA 35 according to method 8.2.2 EBC standard methods (Analytica 2018). The honey wort was transferred for fermentation in a previously washed and disinfected fermenter of the German company "Spidel" with a volume of 30 L. The pure yeast strain was directly sprinkled in to the honey wort according to the manufacturer's instructions. The fermenter was placed in a refrigerator, so the fermentation temperature was maintained at 25°C. The fermentation process was monitored daily and the residual wort extract was checked periodically. At the end of the alcoholic fermentation, lasting 30 days, the mead had an alcohol content of 7%, determined by method 9.2.1 according to EBC standard methods (Analytica 2018).

Once the fermentation had finished (unchanged density and residual sugar <5 $g.L^{-1}$), the mead was cool down till 2°C. All meads were force carbonized using CO_2 pressure. After that 1% of the beehive products were added to each test sample. The experiment was conducted with one control (C) and 3 experimental samples (MPP, MPO, MBB). 1% ethanol extract of propolis (70% v/v) was added to sample MPP. MPO and MBB meads were prepared with addition of 1% pollen and 1% bee bread, respectively.

pH determination. The pH value of the mead samples was measured directly with a pH-meter MS 2004 (Microcyst Ltd, Plovdiv, Bulgaria), equipped with a combined pH electrode S 450 CD (Sensorex pH Electrode Station, Garden Grove, CA, USA).

Colour properties. The mead colour was measured with a Konica Minolta model CR-410 chromameter using the CIE L*, a*, b* system. The calibration step was done with a white reference standard no.18833116 (Y = 94.3, x = 0.3134 and y = 0.3197). The components of colour are: brightness of the colour L* (ranging from 0 black to 100 white): the red component of the colour a* (varying from - green to + red), the yellow component of the colour b* (varying from - b blue to + b yellow), C* – chroma of the colour and *h* – hue angle. Above mentioned components were measured at the following settings: aperture = 8 mm, standard observer 2° and light source D65. The total colour difference (ΔE) (Koren et al. 2020) was also calculated by equation 2:

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$

Extraction and determination of phenolic compounds. Extraction of Phenolic Compounds from mead was obtained according to procedure described by Shopska et. al (2022) with methanol dilution followed by filtration. The methanolic extracts were used for determination of phenolic compounds concentration and antioxidant activity of mead.

Determination of phenolic compounds content. The total phenolic compounds content was determined according to Shopska et. al (2022) with Folin-Ciocalteu (FC) Reagent through mixing 1 mL of methanol extract of mead with 4 mL of FC reagent (10 times diluted with distilled water), and 5 mL of sodium carbonate (7.5%, w/v). After 1 h the absorbance (A) was recorded at 765 nm using UV-VIS spectrophotometer (Camspec Ltd. UK). The results were presented as mg Gallic acid equivalent (GAE).L^{-1} mead: TPC = (A765 + 0.0083) KP 0.0098, mg GAE.L^{-1} (1) where: A765 – absorbance of the sample of 765 nm, Kp – dilution coefficient.

Content of phenolic compounds by the glories method. The content of total phenols, phenolic acids and flavonoids was determined by a modified Glories method (Mazza et al. 1999) at three wavelengths - 280, 320 and 360 nm using UV-VIS spectrophotometer (Camspec Ltd. UK). One mL of methanol extract was mixed with 1 mL of 0.1% HCl in ethanol 95% v/v, 18.2 mL of 2% HCl v/v and the absorbance was measured after 15 min. The results were presented as gallic acid equivalent (GAE.L^{-1}) for TPC, caffeic acid equivalent (CAE.L^{-1}) for PA, and Quercetin equivalent (QE.L^{-1}) for F (Shopska et al. 2022).

FRAP assay. The ferric reducing antioxidant potential (FRAP) was based on the reduction of Fe^{3+} to Fe^{2+} in an acidic medium and the formation of the coloured complex of ferro-tripyridyltriazines. The FRAP assay was performed according to Benzie and Strain (1996) with some modifications from Dinkova et al. (2014). The FRAP reagent was

prepared by mixing 2.5 mL of a solution of TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) (10 mmol.L^{-1}) in 40 mmol.L^{-1} HCl, 2.5 mL aq. $FeCl_3$ solution (20 mmol.L^{-1}) and 25 mL of acetate buffer (0.3 mol.L^{-1}, pH 3.6). In the UV-macro cuvette, 250 µL of tested methanol solution and 2250 µL of FRAP reagent were mixed. After 4 min stay in the dark at room temperature the absorbance was measured at 593 nm against blank in which the extract was replaced by pure methanol. The results were expressed in µmol TE.L^{-1} (Trolox Eq).

AOA by the ABTS (2,20-azinobis- (3-ethylbenzothiazoline-6-sulfonate)) method. The ABTS analysis was performed as described by Shopska et al. (2022). The absorbance was measured at 734 nm against blank with methanol and the results were expressed as µM TE.L^{-1} mead.

Cupric reducing antioxidant capacity method (CUPRAC). The CUPRAC analysis was performed as described by Shopska et al. (2022). The methanol extracts (0.5 mL) were mixed with 1 mL of 0.01 M $CuCl_2.2H_2O$, 1 mL of ammonium acetate buffer (pH 7), 1 mL of 7.5 × 10^{-3} M ethanol solution of neocuproine, and 0.6 mL of distilled water. After 30 min the absorption was measured at 450 nm. The results were expressed as µM TE.L^{-1} mead.

Hydroxymethyl-2-furfural (HMF) content. The determination was performed following the methodology described in AOAC 980.23-1983. Briefly, 5 mL of mead were diluted by 25 mL of distilled water in 50 mL volumetric flask. A 0.5 ml of Carrez I solution were added, mixed, followed by 0.5 mL of Carrez solution II, mixed and diluted to 50 mL with distilled water. Filtered through filter paper, discarding the first 10 mL of filtrate. Five millilitres of filtrate are mixed with 5.0 mL 0.2% $NaHSO_3$ solution (reference). Another 5.00 mL of filtrate are mixed with 5.0 mL distilled water (sample). Tubes are mixed using vortex and absorbance of the samples is measured against the reference at 284 and 336 nm. The HMF concentration is calculated by equation 1:

$$\frac{(A_{284} - A_{336}) \times 14.97 \times 5}{g\ of\ sample}, mg\ HMF /100\ mL\ mead$$

Factor = 14.94 = (126/16.830) ×1000/10) × 100/5)

Where: 126 – molecular weight of HMF; 16.830 – molar Abs of HMF at 284 nm.

Sensory evaluation of the mead. The sensory evaluation of the meads was performed by a panel group of nine trained tasters aged 30–50 years (four women and five men) with experience in wine and beer tasting and trained in mead evaluation. Sensory analysis was performed in a controlled lighting environment, in the tasting room.

The meads were tested in transparent 50 mL tasting glasses covered with watch glass to minimize the evaporation of volatile compounds, at a temperature of 10 – 12°C and in accordance with the methodology described in ISO 6658:2017.

The tasting committee individually evaluated the characteristics: appearance (turbidity/clarity and colour, carbon dioxide release), aroma (intensity and harmony) and taste (intensity and balance), as well as overall impression, aftertaste (length, intensity, balance) of the product. Each criterion is rated on a five-point scale from 0 to 5 according to increasing intensity. When evaluating the aroma and taste, honey evaluation criteria were used, such as the presence of plant notes (shrubs, trees, resin, propolis), floral notes (flowers and fruits), fresh notes (mint, citrus, eucalyptus) and negative smell (rotten, oxidized, mouldy, stable), specific smell and taste from the added beehive products (propolis, pollen, bee bread).

Statistical analysis. Results are presented as Means ± SEM (Standard error of means), where each measurement was repeated 5 times (n=5). ANOVA: Single factor procedure was performed to establish the significance ($p \leq 0.05$) of the added bee product extracts (Bertinetto et al. 2020). The superscripts $^{a, b, c, d}$ indicate significant difference ($p \leq 0.05$).

Results and Discussion

pH value. The pH value depends on the botanical origin of the honey. Previous studies have found that pH in floral honey mead is 2.74, in manna honey mead is 3.10 and in buckwheat mead is 3.23 (Pereira et al. 2019). The pH value of the mead is closely related to the fermentation conditions. The indicator is usually monitored to track the fermentation progress. Acidity plays a significant role in alcohol beverages and impact on their taste and stability (Aleksandar et al. 2021). The results are shown in Table 1. After completion of fermentation and addition of beehive products, the pH range between 3.58±0.05 and 3.65±0.05.

Compared to the control C, the addition both to three beehive products – propolis, pollen and bee bread decrease significantly with 0.7/0.8 points ($p \leq 0.05$) pH in MPP, MPO and MBB mead. the reported decrease in values is not very large, confirming the pH of mead in other studies (Pereira et al. 2019; Aleksandar et al. 2021). a strong decrease was reported in mead fermented with blackberry juice by Aleksandar et al. (2021).

Table 1. Technological properties

		Meads with added bee product extracts		
	Control (C)	**Bee bread (MBB)**	**Bee pollen (MPO)**	**Propolis (MPP)**
pH, (-)	3.65a±0.02	3.59b±0.02	3.58b±0.04	3.58b±0.04
L*, (-)	62.23a±0.74	47.74b±0.23	47.90b±0.72	45.83c±0.36
a*, (-)	-0.34d±0.18	3.91a±0.08	3.23b±0.20	2.49c±0.19
b*, (-)	31.02a±0.26	24.48b±0.46	23.89b±0.53	14.28c±0.35
C*, (-)	31.03a±0.23	24.80b±0.30	24.11b±0.32	14.50c±0.35
H, (-)	90.62a±0.26	80.94c±0.21	82.28b±0.36	80.11d±0.34
ΔE, (-)	-	16.45b	16.40b	23.60a
HMF, g.kg-1	1.43a±0.18	1.43a±0.34	1.05b±0.32	0.60c±0.43

*The superscripts ^{a,b,c,d} indicate significant difference ($p \leq 0.05$).

Instrumentally evaluated colour characteristics. Control mead (C) is light in colour ($p \leq 0.05$) established by the higher L* values (Table 1). MPP, MPO and MBB were 30% darker ($p \leq 0.05$). Three test samples MPP, MPO and MBB differ significantly ($p \leq 0.05$) in colour redness (a*) compared to C. The yellow component (b*) of colour in the mead with added propolis extract (MPP) was two-times lower compared to the control (C). In the same time the addition of pollen and bee bread also decrease significantly b* component of the colour ($p \leq 0.05$), but in smaller manner. The beforementioned trend was also evaluated in chroma (C*) and hue angle (h) values. Both parameters decreased with the addition of the bee product extracts. The hue angle values potentially decrease due to the formed cloudiness in the mead and observed decreased transparency (Fig.1).

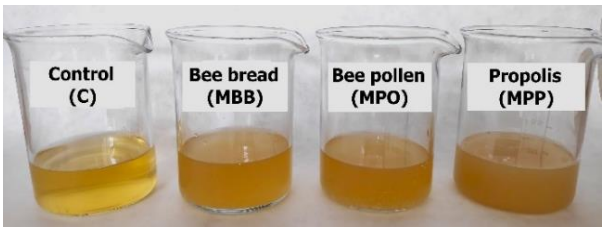


Figure 1. Mead samples

HMF. HMF values range between 0.60 and 1.43 $mg.kg^{-1}$. HMF values in control C, MPO and MBB do not differ significantly ($p \geq 0.05$). Compared to C, two-time lowest ($p \leq 0.05$) HMF content was established in propolis mead (MPP). In all studied samples HMF values were below 40 $mg.kg^{-1}$ according to Bulgarian legislation (Ordinance No. 48 of November 11, 2003. On the procedure and methods for taking samples and the methods used for the analysis of honey). The results are shown in table 1. The honey itself is a source of compounds with antioxidant activity as flavonoids, α-tocopherol, ascorbic acid, β-carotene, catalase and peroxidase (Simão et al. 2023). In study explored commercial meads and soy honey mead Akalın et al. (2017) found that the phenolic content of the honey correlates with antioxidant capacity of mead. Our results show three times higher TPC after propolis addition in mead. Several studies describe strong antioxidant action of propolis related to huge biological active compounds in its composition (Kumar et al. 2021; Kolayli & Keskin 2020). Propolis content nearby 20% polyphenols and is expected that after addition of 1% ethanolic tincture (70% v/v) in MPP mead the highest values of TPC by FC method was established. Close to the control (C) was TPC (FC method) of meads with pollen (MPO) and bee bread (MPP). The phenolic compounds measured by Glories method decreased at the following order: MPP; MPO; MBB; C. The results of TPC measured by FC and Glories methods are different because the data obtained by FC are influenced by the oxidative status of the sample (Shopska et al. 2022).

Table 2. Bioactive composition and activity

	Meads with added bee product extracts			
	Control (C)	**Bee bread (MBB)**	**Bee pollen (MPO)**	**Propolis (MPP)**
TPC*, mg GAE.L^{-1}	653.21^{c}±3.64	612.10^{d}±3.08	695.94^{b}±2.61	1918.22^{a}±3.22
TPC, mg GAE.L^{-1}**	74.59^{d}±1.88	92.71^{c}±3.26	123.74^{b}±11.26	259.16^{a}±20.04
Phenolic acids, mg CAE.L^{-1}	11.91^{d}±0.54	24.14^{c}±0.15	42.94^{b}±3.96	137.32^{a}±12.09
Flavonoids, mg QE.L^{-1}	7.20^{d}±0.22	11.21^{c}±0.45	26.70^{b}±4.57	60.24^{a}±4.57
DPPH, µmol TE.L^{-1}	161.88^{d}±2.00	292.38^{c}±2.84	338.93^{b}±2.13	946.73^{a}±5.22
ABTS, µmol TE.L^{-1}	333.70^{d}±2.10	392.31^{c}±0.77	441.38^{b}±1.15	2730.64^{a}±2.17
FRAP, µmol TE.L^{-1}	184.04^{d}±1.45	228.61^{c}±2.61	410.11^{b}±2.89	3294.55^{a}±3.60
CUPRAC, µmol TE.L^{-1}	505.47^{d}±1.98	618.81^{c}±1.58	678.53^{b}±2.93	3832.22^{a}±3.85

Table 3. Sensory profiles

		Meads with added bee product extracts			
		Control (C)	**Bee bread (MBB)**	**Bee pollen (MPO)**	**Propolis (MPP)**
Appearance	Colour	Gold	Gold	Gold	Gold
	Transparency	Hazy - 3	Hazy - 3	Hazy - 3	Hazy - 4
Aroma	Intensity	4.20±0.10	5.00±0.10	4.90±0.10	3.00±0.12
	Harmony	4.00±0.12	4.90±0.20	4.50±0.20	2.00±0.15
Taste	Intensity	3.50±0.42	5.00±0.00	4.70±0.15	2.00±0.15
	Balance	3.60±0.45	4.90±0.10	4.80±0.10	2.50±0.10
	Acidity	4.00±0.05	4.00±0.10	4.00±0.10	4.00±0.05
	Density	4.00±0.15	5.00±0.10	4.90±0.10	3.00±0.15
Overall experience	Balance	4.00±0.20	5.00±0.10	4.90±0.10	2.50±0.10
	General quality	3.90±0.25	4.83±0.37	4.63±0.34	2.75±0.75
Specific smell and taste from the added beehive products		none	none	none	Medicine

Antioxidant capacity. The antioxidant potential of mead with added beehive products measured with different methods is important for confirmation of high biological value of the beverage. The results for AOA evaluated by DPPH, FRAP, ABTS, CUPRAC and ORAC are shown in Table 2. The lowest AOA was measured by the DPPH method, and the highest by the CUPRAC and FRAP methods. The lowest antioxidant activity measured by four used methods (DPPH, ABTS, FRAP,

CUPRAC) was established in control C, followed by mead with bee bread (MBB) and pollen (MPO). However, the DPPH values in MBB and MPO was 1.8 ($p \leq 0.05$) and 2 ($p \leq 0.05$) times higher compared to C. ABTS results show 1.17 ($p \leq 0.05$) and 1.32 ($p \leq 0.05$) times increase after 1% bee bread and pollen addition. Mead produced by 1% propolis extract (70% v/v ethanol) addition displayed the highest antiradical activity (DPPH) and AOA measured by ABTS. The results for the highest antioxidant activity of MPP mead were expected because of the highest TPC, phenolic acids and flavonoid content Moreover, they confirmed previously studies that the increase in AOA corresponded to the increase in biological active components of mead (Akalın et al. 2017).

Conclusions

Propolis (1%), pollen (1%) and bee bread (1%) decrease significantly pH values of the mead. Beehive products increase significant antioxidant activity (DPPH ABTS FRAP and CUPRAC) TPC, phenolic acids and flavonoid content with strongest manifestation in mead supplemented with propolis. Bee pollen and bee bread give harmony test and smell in mead. Propolis in addition of 1% leads strong intrusive taste identical to medicine. According to our results is recommended the use of propolis below 1% in mead. The use of beehive products as supplements in mead is appropriate for processing of innovative value-added belvedere.

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