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Original Research

The Effect of Storage of Bee Pollen Extracts on Polyphenol Content

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Abstract

Bee pollen belongs to bee products that are characterized by high nutritional value and biotic activity. These characteristics result from the wide variety of compounds that bee pollen contains.

Our study determined the effects of storage conditions of bee pollen extracts on polyphenol content. The study was conducted with the use of three types of bee pollen extracts, namely ethanol extracts, enzymatic hydrolysates from pollen, and ethanol extract of pepsin-digested bee pollen. Polyphenol content in the studied extracts was determined immediately after extraction and after 12-month storage. We have concluded that 12-month storage of bee pollen extracts decreases polyphenol concentration in all three types of extracts, and the changes depend on the storage conditions.

Keywords: bee pollen, pollen extracts, polyphenols

Introduction

Polyphenols belong to a large group of organic compounds of varied structure that are present in plants. Their specific chemical structure determines polyphenol biological activity, and the presence of a phenol aromatic ring is their common characteristic. Strong antioxidant properties of polyphenols are closely related to the presence of conjugated double bonds, and the number and location of hydroxyl groups in an aromatic ring [1-3]. Due to their free radicals scavenging activity, polyphenols have a protective function in cardiovascular diseases caused by oxidative stress. Moreover, polyphenols have anti-inflammatory, anti-allergic, antiviral, anticoagulant, anticancerogenic, and immunostimulant properties, and they also act as inhibitors of specific enzymes [4-6].

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Recently, a lot of researchers have focused on the health-promoting and curative effects of natural products. Bee products rank high among dietary supplements, and bee pollen is included in this group [7, 8]. Bee pollen is a valuable apitherapeutic recognized in the medical, health, and nutrition field. Nutritional values of bee pollen result from the fact that it contains proteins, amino acids, carbohydrates, lipids (omega-3 and omega-6 fatty acids), vitamins, and minerals [9-11]. Bee pollen therapeutic and protective effects is relate to the content of polyphenols, defined as the main components that determine bee pollen antioxidant activity [12-14].

Those above-listed properties of bee pollen to a large extent depend on the plant species it is obtained from, and the properties change during the period of storage. Published data suggest that bee pollen storage significantly decreases its antioxidant activity, and in consequence it affects bee pollen products [15]. Thus the aim of our study

was to determine the effect of storage conditions of bee pollen extracts on polyphenol content.

The study aim was achieved by determining polyphenol content in three types of bee pollen extracts, namely ethanol extracts, enzymatic hydrolysates from pollen, and ethanol extract of pepsin-digested bee pollen. Determination was carried out immediately after obtaining the extracts and after 12-month storage under various conditions (at 4-8ºC in the dark, at room temperature of ~25ºC in the dark, and at room temperature of ~25ºC in light).

Material and Methods

The study material was comprised of three types of bee pollen extracts, i.e. ethanol extract of bee pollen (EEP), enzymatic hydrolysates from pollen (PEP), and ethanol extract of pepsin-digested bee pollen (EEPP). The material used for extracts was ground bee pollen collected in 2008 in the apiary called "BARĆ," named by priest dr Henryka Ostacha in Kamianna, Poland.

Ethanol extracts (EEP) were prepared according to a slightly modified method of Almaraz-Abarca et al. [16]. The ethanol extract of bee pollen was prepared by weighing 20 g of ground bee pollen, with accuracy of 0.01 g. Then the bee pollen sample was extracted 5 times with 50% (v/v) ethanol aqueous solution, in 200 cm^3 portions, and shaken each time for 60 min at room temperature, in order to macerate the sample. After each extraction, the sample was filtered under reduced pressure with the use of a water pump. The filtrate was collected, and substrate was extracted again with another portion of ethanol. The obtained filtrate was centrifuged at 10,000 rpm for 10 min, and then it was evaporated under reduced pressure in a rotary vacuum evaporator (UNIPAN-PRO 350P). The evaporated extract was dried in a laboratory incubator at 38ºC to obtain solid mass. The extract was weighed and then dissolved in 50% (v/v) ethanol aqueous solution to obtain a concentration of 2 mg/cm^3. These extracts are further referred to as EEP.

Enzymatic hydrolysates from pollen (PEP) were prepared according to a slightly modified method described by Nagai et al. [17]. 20 g of bee pollen was weighed. Then the sample was mixed with distilled water acidified with concentrated HCL to pH=2. The volume of distilled water was 5 times the volume of the sample. Pepsin was added to the sample to obtain a concentration of 1.0%. Next the sample was incubated at 37ºC for 48 h. Hydrolysis was arrested by boiling for 10 min. The obtained enzymatic hydrolysates was filtered under reduced pressure with the use of a water pump. Then the filtrate was centrifuged at 10,000 rpm for 10 min, and the supernatant was evaporated in a rotary vacuum evaporator (UNIPAN-PRO 350P). The obtained extract was dried in a laboratory incubator at 38ºC. A dry enzymatic hydrolysates was weighed and then dissolved in acidified distilled water to obtain the concentration of 2 mg/cm^3. This extract was used for further assays and referred to as PEP.

Ethanol extracts of pepsin-digested bee pollen (EEPP) were obtained in accordance with a method described by Rzepecka-Stojko et al. [18]. The obtained supernatant after pepsin extraction of bee pollen was extracted with 200 cm^3 50% (v/v) of ethanol aqueous solution. The sample was extracted for 60 min at room temperature and frequently shaken. The extract was filtered under reduced pressure, and then the filtrate was centrifuged at 10,000 rpm for 10 min. The supernatant was evaporated under reduced pressure in a rotary vacuum evaporator (UNIPAN-PRO 350P). Next the extract was dried in a laboratory incubator at 38ºC. The dry extract was dissolved in 50% (v/v) of ethanol aqueous solution to obtain the concentration of 2 mg/cm^3. This extract was used for further assays and referred to as EEPP.

Total polyphenol content in bee pollen extracts was determined spectrophotometrically with the use of the Folin-Ciocalteu reagent (FCR) as described in a study by Singleton et al. [19].

The determination of polyphenol content involves the reduction of a phosphotungstic and phosphomolybdic acid mixture by polyphenols to obtain blue oxides of tungsten and molybdenum. The maximum absorption of the oxides is at λ=760 nm, whereas color intensity is proportional to total polyphenol content in a studied sample [19]. To determine the polyphenol concentration, each studied extract was diluted with 50% (v/v) ethanol aqueous solution or distilled water acidified with concentrated HCl to pH 2 to obtain the concentration of 2 mg/cm^3.

Next, disposable test tubes were filled with 2 cm^3 of extract to which 2 cm^3 of Folin-Ciocalteu reagent was added, and the test tubes were left for 5-8 min. After 5-8 min, 2 cm^3 of 10% sodium carbonate solution was added, and the samples were incubated for 1 h at room temperature. Then the absorbance of each sample was assayed spectrophotometrically at a wavelength of λ=760 nm. A standard sample was Folin-Ciocalteu reagent, to which 10% sodium carbonate and 50% (v/v) aqueous ethanol solution or distilled water of pH 2 was added.

Two samples from each type of extract were prepared for the study: ethanol extract of bee pollen (EEP), enzymatic hydrolysates from pollen (PEP), and ethanol extract of pepsin-digested bee pollen (EEPP). Polyphenol concentration was determined immediately after obtaining extracts. For each sample we carried out 2 sets of assays, and each set was repeated 3 times.

To determine the effects of bee pollen storage conditions on polyphenol content, 3 samples were collected from each studied extract and then left for 12 months under various storage conditions: at 4-8ºC in the dark, at room temperature in the dark, and at room temperature in light. After 12 months, polyphenol concentration was determined as described above, and compared with earlier results. Polyphenol concentration was calculated on the basis of a gallic acid standard curve within concentrations of 0.00-0.15 mg/cm^3 and expressed as mg/1 cm^3 of extract. Next, the polyphenol concentration was recalculated in relation to extract weight and reported in gallic acid equivalents, i.e. mgGAE/g of extract. Likewise, polyphenol content in fresh extracts was expressed, and polyphenol content in relation to bee pollen weight was calculated additionally, and reported in mgGAE/g of bee pollen.

Table 1. The polyphenol content in studied extracts of bee pollen fresh and stored.

		Fresh extracts	Extracts stored for 12 months		
			4-8ºC in the dark	Room temperature in the dark	Room temperature in light
Polyphenol content in extracts (mg GAE/g) (+SD)	EEP (n=12)	21.30 (+0.42)	17.05 (+0.92)	13.45 (+0.21)	12.30 (+0.28)
	PEP (n=12)	14.95 (+4.59)	12.10 (+4.38)	10.55 (+3.46)	8.80 (+1.84)
	EEPP (n=12)	39.95 (+2.33)	36.80 (+1.13)	34.55 (+0.49)	31.25 (+1.91)
Decreases in concentrations of polyphenols in storage extracts (mg GAE/g)	EEP	-	4.25	7.85	9.00
	PEP	-	2.85	4.40	6.15
	EEPP	-	3.15	5.40	8.70

GAE – gallic acid equivalents

Results and Discussion

The polyphenol content assay employed in our study is commonly used to assay these compounds in natural products and bee products, including bee pollen [4, 12, 17].

The average polyphenol concentration in fresh samples of ethanol extracts (EEP) was 21.3 mgGAE/g of extract (Table 1). Published value for this extract, i.e. 24.6 mgGAE/g [4], 12.4 mgGAE/g [5], or 32.4 mgGAE/g [18]. Average polyphenol content in relation to bee pollen weight was 11.3 mgGAE/g. Published data show that polyphenol content in bee pollen is rather varied, and according to different researchers, the values for multiflower pollen are 8.2 mgGAE/g [4] or 30.46 mgGAE/g [20], respectively. After the analysis of polyphenol concentration in ethanol extracts of bee pollen (EEP) that were stored for 12 months under various conditions, it was concluded that the biggest difference between polyphenol concentrations was recorded in the extracts stored at room temperature in the light. The polyphenol concentration in these extracts was 12.3 mgGAE/g (Table 1), which was 57.7% of polyphenol content in fresh extracts (Fig. 1). The polyphenol content in ethanol extracts (EEP) stored for 12 months at room temperature in the dark was 13.45 mg/g of extract (Table 1), which was 63.1% of the polyphenol content in fresh extracts (Fig. 1).

On the basis of the conducted study results, we found that the smallest changes in polyphenol concentrations were recorded in extracts stored at 4-8ºC. The average polyphenol content in these extracts (EEP) was 17.05 mgGAE/g of extract (Table 1), which was 80.0% of the initial polyphenol content in fresh extracts (Fig. 1).

The average concentration of polyphenols determined in fresh samples of pepsin hydrolysates from pollen (PEP) was 14.95 mgGAE/g of extract (Table 1), while the published value of the total content of polyphenols in this type of extract was 10.39 mgGAE/g [17]. Polyphenol content in relation to bee pollen weight was 10.55 mgGAE/g.

On the basis of the obtained results for pepsin hydrolysates from pollen (PEP) after 12-month storage under various conditions, we found that the biggest difference between the polyphenol concentrations was characterized by

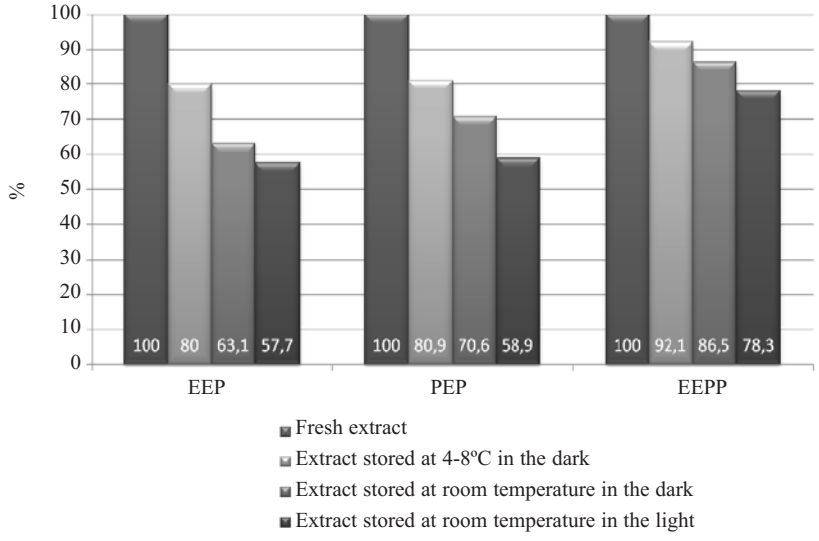

Fig. 1. Polyphenol percentage content in the studied extract: EEP – ethanol extract, PEP – pepsin hydrolysates, EEPP – ethanol extract of pepsin-digested bee pollen. The polyphenol concentration in fresh extracts has been assumed to be 100%.

the extracts stored at room temperature in light. The concentration of polyphenols in these extracts was 8.8 mgGAE/g (Table 1), which was 58.9% of polyphenol content in fresh extracts (Fig. 1). The concentration of polyphenols in PEP stored for 12 months at room temperature in the dark was 10.55 mgGAE/g of extract (Table 1), i.e. 70.6% of polyphenol content in fresh extracts, which was assumed to be 100% (Fig. 1).

On the basis of the study results, we found that the smallest changes in the concentrations of polyphenols were recorded in pepsin hydrolysates from pollen (PEP) stored at 4-8ºC. Average polyphenol content in these extracts was 12.1 mgGAE/g of extract (Table 1), which was 80.9 % of the initial polyphenol content in fresh extracts (Fig. 1).

The analysis of results obtained for samples of ethanol extracts of pepsin-digested bee pollen (EEPP) was carried out in a similar manner. After absorbance measurements we calculated the content of polyphenols in mg/1 cm^3 in the extract of 2 mg/cm^3 concentration. The determined concentration of polyphenols in fresh ethanol extract of pepsin-digested bee pollen (EEPP) was 39.95 mgGAE/g of extract, which was presented in Table 1.

We could evaluate the effect of bee pollen storage on the polyphenol concentration on the basis of obtained results. After 12 month-storage of ethanol extracts of pepsin-digested bee pollen (EEPP) under various conditions, the biggest difference was recorded for the extracts stored at room temperature in the light. The average polyphenol concentration in these extracts was 31.25 mgGAE/g (Table 1), which was 78.3% of polyphenol content of fresh extracts (Fig. 1). The concentration of polyphenols in EEPP stored for 12 months at room temperature in the light was 34.55 mgGAE/g of extract (Table 1), i.e 86.5% of polyphenol content of fresh extracts, which was assumed to be 100 % (Fig.1).

After the analysis of obtained results, we concluded that the smallest changes of polyphenol concentration were recorded in EEPP stored at 4-8ºC. The content of polyphenols in these extracts was 36.8 mgGAE/g of extract (Table 1), which was 92.1% of the initial content of polyphenols in fresh extracts (Fig.1).

Conclusions

It was established that polyphenol content of the extracts was related to the extraction method. The highest concentration of polyphenols was determined in ethanol extracts of the precipitate formed after earlier pepsin hydrolysis, whereas the lowest one was characterized by enzymatic hydrolysates from pollen. Ethanol extraction following pepsin hydrolysis is the most effective method of polyphenol extraction.

To conclude, we can say that 12-month storage of bee pollen decreases the concentration of polyphenols in all three types of extracts, and these changes depend on the storage conditions. The biggest decrease in the polyphenol concentration was recorded in all types of extracts stored at room temperature in light, while, storing at 4-8ºC in the dark was the best storage condition for all types of extracts. The pepsin-digested ethanol extracts of bee pollen (EEPP) were characterized by the highest content of polyphenols after storage.

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