STUDY ON THE DYNAMICS OF SOME BIOACTIVE ELEMENTS FROM BEE POLLEN EXTRACTS

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Abstract

Analyses carried out have followed the determination of the amount of total polyphenols, flavanols, total flavonoids and antioxidant activity of bee pollen extracts harvested by collectors from Moldova. These determinations were performed according to different conditions: time, temperature and light. Two methanolic extracts have been used different in the methanolic solvent concentration used: 96% and 80%. Analyses were performed on fresh pollen extract, kept a week and kept two weeks, at 4°C, 20°C, the presence or absence of natural light. We have detected fluctuations in the quantities of compounds followed, both in relation to storage conditions and depending on the solvent used. The amounts of total polyphenols detected had mean values between 27.13 and 29.90 mg GAE / g pollen for extract 1 (solvent methanol concentration 96%) and between 23.56 and 27.67 mg GAE / g pollen for extract 2 (solvent methanol concentration 80%). Quantities of flavanols values were between 8.93 and 7.43 mg QE / g pollen (extract 1), and between 4.95 and 5.92 mg QE / g pollen (extract 2), describing a general downward dynamic. Total flavonoids recorded were between 20.44 and 28.00 mg QE / g pollen (extract 1), and between 16.17 and 19.50 mg QE / g pollen (extract 2). The antioxidant activity determined was between 24.18 and 14.24% inhibition (extract 1), and between 26.71 and 19.90% inhibition (extract 2).

Key words: pollen extract, dynamic, polyphenols, flavonoids, antioxidant activity

INTRODUCTION

Pollen collected by bees is an excellent remedy for various diseases, while representing a balanced and nutritious food. Biochemical composition of pollen varies from one species to another. [18]. At the same time, its quality is dependent on several factors such as packaging, conditioning and storage methods. Studies claim that the polyphenols present in pollen (flavonoids, phenolic acids) determines in large part its antioxidant capacity [4, 10, 16]. Due to its bioactive effects bee pollen is important both for food and pharmaceutical industry. One of the used forms of the compounds in the pollen extracts, are made in alcoholic solvents. Some data [13] show higher levels of polyphenols detected in alcoholic extracts than in fresh pollen and an increase in antioxidant activity. However, the detection methods efficienty are different [17]. Studies of content in bioactive components of monofloral and multifloral pollen [14], are reduced in number. Analyses performed in this study follow the dynamics of bioactive elements from bee pollen extracts (total polyphenols, flavanols, total flavonoids, antioxidant activity), kept under different conditions of temperature and light. There are few studies on the dynamic properties of bee products [1]. In research, the used extracts had two different concentrations of methanolic solvent (96% şi 80%). Analyses were performed on fresh extract, kept one week and two weeks.

MATERIAL AND METHOD

A quantity of 200 g fresh pollen was ground into a fine powder. 40 g powder has been weighted and placed in a 150 ml Berzelius glass with 50 ml methanol 96%. The mixture was stirred at ultraturax (Heildolph DIAX 900) for 5 minutes. Subsequently, the mixture was placed in a water bath ultrasonicator (Super RK Sonorex Bandelin 100H) for 30 minutes. The next

step was the stirring of the extract on a magnetic stirrer (15 Laborgerateborse Poly Variomag GmbH) for 60 minutes. The entire amount of extract and residue was placed in centrifuge tubes with lids and centrifuged at 3000 rpm for 20 minutes in a centrifuge (Sigma 25). The supernatant was collected in an Erlenmeyer flask with glass stopper, and over the residue was added a fresh portion of 50 ml 96% methanol. The re-extraction was performed by stirring on a magnetic stirrer for 60 minutes, repeating this operation three times further. Finally, the combined supernatants were brought to a concentration of 10% (to the initially 40 g of pollen, were added a total of 560 ml methanol). The same operations were carried out for the second extraction solvent (methanol 80%).

Two extracts of 10% concentration were obtained (96% and 80% refers to the concentration of extraction solvent, and 10% refers to the concentration of extract). These two extracts were subjected to chemical analysis: determination of total polyphenols, flavanols determination, the determination of total flavonoids, determination of anti radical activity. It was noted that 10% extract is highly concentrated in the biologically active principles, the reason for the above analysis was done by diluting the extract concentration of 1% for polyphenols and flavonoids and 0.1% for the anti radical activity. Four extracts were obtained with the following characteristics: 10% pollen extract in 96% methanol; 10% pollen extract in 80% methanol; 1% extract (from 10%) of pollen in 96% methanol; 1% extract (from 10%) of pollen in 80% methanol.

The second stage of the experiment was the keeping of the extracts, under different conditions, at dark and 4°C (refrigerator); at natural light and to 25°C (room temperature), at dark and 25°C (room temperature). Extracts were made in two different concentrations, used to observe its influence on the content of active principles in different storage conditions.

To determine the total polyphenol content the Folin-Ciocalteu method was used [9], taken and modified by various authors, adapted for all types of matrices which are to determine the total polyphenol content [8, 11, 12, 19]. In order to determine the quantities of polyphenols in pollen samples, a calibration curve was made with known concentrations of gallic acid [15] and unknown sample absorbance measurement was performed with the pollen calibration curves. Results were expressed as mg GAE/g of pollen (mg gallic acid equivalents per gram of pollen).

Analysis of flavanols and total flavonoids was reported by various authors who used different methods with specific reagents.

Dowd method [22], retrieved and modified by various authors [2, 12, 15], is using a solution of aluminum chloride as a specific reagent, which reacts with the flavonoids present in the sample, giving a yellow color, its intensity beeing determinated spectrophotometrically at 415 nm.

Extraction of flavanols: from the 1% pollen extract 2 ml were taken (equivalent to 0.02 g of pollen) and were mixed with equal quantity of a solution of 2% aluminum chloride in methanol. After 10 minutes, the pollen sample absorbance has been read at 415 nm with a spectrophotometer in comparison to the witness sample containing 2 ml pollen extract and 2 ml methanol. Extraction of total flavonoids: 1 ml pollen extract 1% (equivalent to 0.01 g of pollen) was mixed with 0.3 ml of 5% $NaNO_2$. After five minutes 0.3 ml $AlC1_3$ 10% were added Samples were shaken, and after another six minutes were neutralized with 2 ml NaOH 1M. Absorbance reading was achieved using the calibration curve. Results were expressed as the average of three repetitions in mg quercetin equivalents per g pollen (mg QE / g pollen).

The method used by some authors [3, 6] for measuring antioxidant activity was adapted [21] after the original method of Brand-Williams [5] and was followed in this study. The DPPH method is based on measuring the ability of antioxidants to block the radical 2,2-diphenyl-1-picril-hidrazil. The dark blue DPPH free radical, is reduced to the hydrazine fadeing when reacts with different hydrogen donors. This ability is measured using ultraviolet spectrophotometry, based on that signal intensity after the reaction is inversely proportional to the antioxidant concentration and with the reaction time, the absorbance

change beeing monitored at 517 nm. To prepare samples for analysis two extraction solvents have been used : methanol 96% and 80% methanol. These were mixed with 2.5 ml of DPPH solution concentration 0.03 mg / ml in methanol. There followed a phase of agitation and incubated at room temperature and darkness for 15 minutes then remained consumed DPPH solution absorbance was determined at 517 nm against blank.

Experimental groups were organized as follows: E1 - pollen extract fresh E2 - pollen extract stored 7 days at 4°C in the absence of light, E3 - pollen extract stored 7 days at 22°C and natural light, E4 - pollen extract stored 7 days at 22°C absence of light, E5 - pollen extract stored 14 days at 4 ° C in the absence of light E6 - pollen extract stored 14 days at 22°C and natural light, E7 - pollen extract stored 14 days at 22°C in the absence of light.

RESULTS AND DISCUSSIONS

The amounts of total polyphenols, detected in extracts of fresh pollen had averages of 27.64 mg GAE / g pollen (extract 1) (Table 1) respectively 23.56 mg GAE / g pollen (extract 2) (Table 2). Higher concentration of methanol led to a more accurate detection or a increased release in the amount of total polyphenols. Extract 1, kept at refrigerated temperature (4°C) presented a dynamic upward, detecting the average value of 27.33 mg GAE / g pollen after the first week and 29.90 mg GAE / g pollen after the second week (Fig. 1).

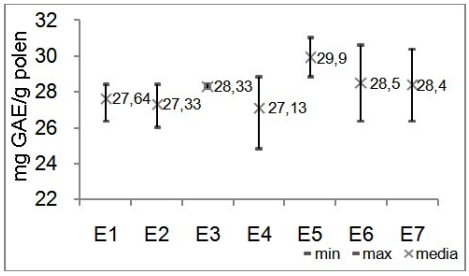

Figure 1. Dynamics of total polyphenols, extract 1

In the extract 2, total polyphenol values detected in the refrigerated samples were 27.67 mg GAE / g pollen after the first week and 27.60 mg GAE / g pollen after the second week (Fig. 2). Lots kept at 22°C and daylight showed an upward dynamic in the case of extract 1 (E3 28.33 mg GAE / g pollen and E6 28.50 mg GAE / g pollen) (Fig. 1). Extract 2 values for the same two conditions were E3 26.87 mg GAE / g pollen and E6 26.80 mg GAE / g pollen. Extracts kept at 22°C in the absence of natural light had values of E4 27.13 mg GAE / g pollen and E7 28.40 mg GAE / g pollen for extract 1; E4 26.80 mg GAE / g pollen and E7 26.10 mg GAE/g pollen extract 2.

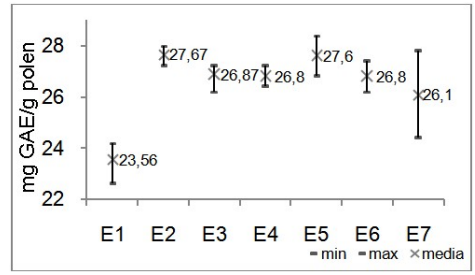

Figure 2. Dynamics of total polyphenols, extract 2

Average amounts of flavanols, recorded for fresh pollen extracts had values of 8.57 mg QE / g pollen for extract 1 (Table 1) and 5.92 mg QE / g pollen for extract 2 (Table 2). There is a greater amount of flavanols detected when the methanolic solvent of the extract was 96%. Extract 1, kept at 4°C in the absence of light, showed a slight decrease in the quantity of flavanols, after the first week of storage, up to the average value of 8.33 mg QE / g pollen (Fig. 3). After the second week was an upward growth, registering an average of 8.93 mg QE / g pollen. In terms of extract 2, kept under the same conditions, both values fluctuated downward after the first week (E2 5.65 mg QE / g pollen), and after the second week (E5 5.38 mg QE / g pollen) (Fig. 4).

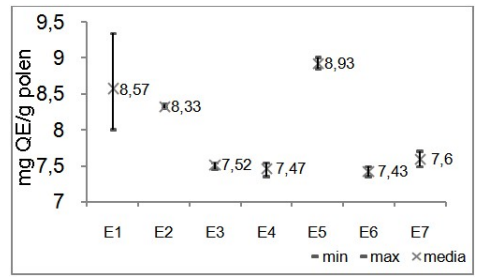

Figure 3. Dynamics of flavanols, extract 1

Extracts kept at a temperature of 22°C showed a downward dynamic for lots held at natural light (E3 7.52 mg QE / g pollen E6 7.43 mg QE / g pollen) for extract 1 the values found beeing of E4 7.47 mg QE / g pollen and E7 7.60 mg QE / g of pollen for lots held in the absence of light (Fig. 3).

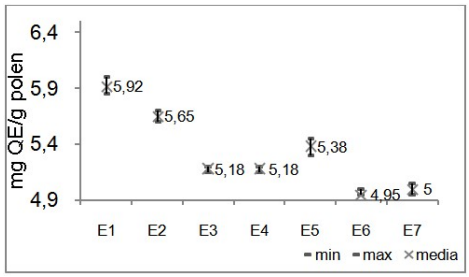

Figure 4. Dynamics of flavanols, extract 2

For extract 2, the dynamic decreased for all lots stored at 22°C (E3 5.18 mg QE / g pollen E6 4.95 mg QE / g pollen), (E4 5.18 mg QE / g pollen E7 5.00 mg QE / g pollen) (Fig. 4). The highest amount of flavanols found (reported to the fresh pollen extract lots), was detected useing the 96% methanolic solvent, for the samples stored at refrigeration temperature after two weeks. For the extracts made with 80% methanol solvent, the dynamics for all conditions was a descendant one.

Quantities of total flavonoids showed upward dynamics for all storage conditions, for extract 1 (Table 1, Fig. 5) and for most storage conditions, for extract 2 (Table 2, Fig. 6). Thus, the values recorded for fresh pollen extracts were of 20.44 mg QE / g pollen in extract 1 and 16.52 mg QE / g pollen in extract 2. As with flavanols, total flavonoids had higher average value achieved when the methanol solvent in extract had a higher concentration (96%).

For extract 1 stored at 4°C in the absence of light, the values dynamic has been a rising one, detecting values of 20.47 mg QE / g pollen after the first week, and 28.00 mg QE / g pollen after the second week. When kept at 22°C, the dynamic was slightly downward (E3 27.90 mg QE / g pollen E6 27.80 mg QE / g pollen), (E4 26.73 mg QE / g pollen E7 21 , 65 mg QE / g pollen) (Fig. 5).

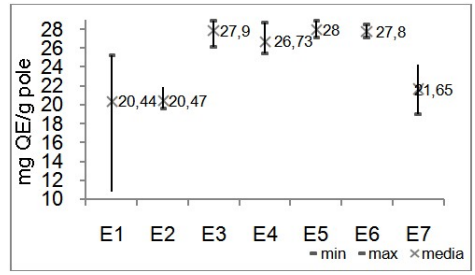

Figure 5. Dynamics of total flavonoids, extract 1

For the extract stored at 4°C in the absence of light, the dynamics was a increasing , detecting the values of 16.90 mg QE / g pollen after the first week, and 18.05 mg QE / g pollen, after week second. When extracts were kept at 22°C, the growth was a rising (E3 16.83 mg QE / g pollen E6 19.50 mg QE / g pollen), (E4 16.17 mg QE / g pollen E7 19 15 mg QE / g pollen) (Fig. 6).

Analysis of the anti radical activity showed a downward trend of the mean values of inhibition found in extract 1 (Table 1). Thus, from the initial value of 24.18% inhibition, detected for the fresh pollen extract, the means decreased after the first week to 22.65% inhibition for E2, 17.81% inhibition for E3 and 21.22% inhibition for E4. After the second week values continued to decline, except group E6. Mean values found were 19.39% inhibition for E5, 18.18% for E6 and 14.24% for E7.

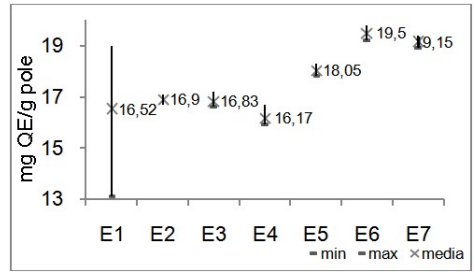

Figure 6. Dynamics of total flavonoids, extract 2

In the case of extract 2, the averages rose from baseline after the first week (Table 2). Thus, from the average value of 24.45% inhibition for the E1 group, values increased to 26.71% inhibition for E2, 26.21% inhibition for E3 , and 26.50% inhibition for E4. Analyses carried out after the second week showed declines in the values of

antioxidant activity. The values were presented as follows: 19.90% inhibition for E5, 20.59% inhibition for E6 and 24.26% inhibition for E7.

Dynamics of the values found for extract 1 (Fig. 7), have revealed that the detection of antioxidant activity recorded decreases in the value of % inhibition, for both the first and after the second week (when the methanolic solvent extract has a concentration of 96%).

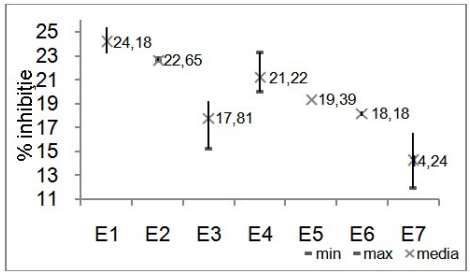

Figure 7. Dynamics of antioxidant activity, extract 1

In the case of extract 2, with a 80% concentration of methanolic solvent (Fig. 8), anti radical activity detected ranged upward after the first week and then decreasing after the second week.

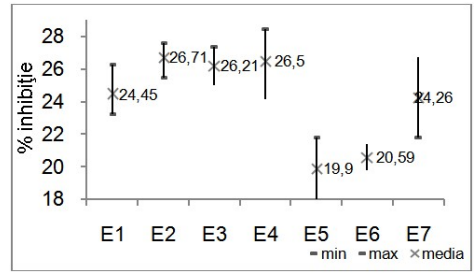

Figure 8. Dynamics of antioxidant activity, extract 2

Table 1. Descriptive statistics of chemical indexes analyzed at extract 1

Lot	Analysis	n	$\bar{x} \pm s_{\bar{x}}$	s	s^2	V%	min	max
E1	T. polyphenols	5	27,64 ± 0,35	0,79	0,628	2,87	26,40	28,40
	Flavons	3	8,57 ± 0,31	0,69	0,482	8,10	8,00	9,35
	Flavonoids t.	3	20,44 ± 2,72	6,08	36,98	29,75	10,80	25,20
	Antioxidant act.	4	24,18 ± 0,51	1,01	1,0217	4,18	23,22	25,41
E2	T.polyphenols	5	27,33 ± 0,71	1,22	1,493	4,47	26,00	28,40
	Flavons	3	8,33 ± 0,02	0,03	0,0008	0,35	8,30	8,35
	Flavonoids t.	3	20,47 ± 0,72	1,25	1,56	6,11	19,60	21,90
	Antioxidant act.	4	22,65 ± 0,08	0,13	0,0176	0,59	22,57	22,80
E3	Polifenoli	5	28,33 ± 0,07	0,12	0,0133	0,41	28,20	28,40
	Flavons	3	7,52 ± 0,03	0,06	0,0033	0,77	7,45	7,55
	Flavonoids t.	3	27,90 ± 0,90	1,56	2,44	5,60	26,10	28,90
	Antioxidant act.	4	17,81 ± 0,99	1,72	2,9426	9,63	15,91	19,24
E4	T.polyphenols	5	27,13 ± 1,20	2,08	4,333	7,67	24,80	28,80
	Flavons	3	7,47 ± 0,06	0,10	0,0108	1,39	7,35	7,55
	Flavonoids t.	3	26,73 ± 1,00	1,74	3,0233	6,50	25,40	28,70
	Antioxidant act.	4	21,22 ± 1,04	1,80	3,2403	8,48	19,95	23,28
E5	T.polyphenols	5	29,90 ± 0,64	1,10	1,21	3,68	28,80	31,00
	Flavons	3	8,93 ± 0,04	0,08	0,0056	0,84	8,85	9,00
	Flavonoids t.	3	28,00 ± 0,52	0,90	0,81	3,21	27,10	28,90
	Antioxidant act.	4	19,39 ±	0,00	0,00	-	19,39	19,39
E6	T.polyphenols	5	28,50 ± 1,21	2,10	4,41	7,37	26,40	30,60
	Flavons	3	7,43 ± 0,04	0,08	0,0056	1,01	7,35	7,50
	Flavonoids t.	3	27,80 ± 0,40	0,70	0,49	2,52	27,10	28,50
	Antioxidant act.	4	18,18 ± 0,12	0,20	0,04	1,10	17,98	18,38
E7	T.polyphenols	5	28,40 ± 1,15	2,00	4,00	7,04	26,40	30,40
	Flavons	3	7,60 ± 0,06	0,10	0,01	1,32	7,50	7,70
	Flavonoids t.	3	21,65 ± 1,53	2,65	7,0225	12,24	19,00	24,30
	Antioxidant act.	4	14,24 ± 1,34	2,33	5,4056	16,32	11,92	16,57

Table 2. Descriptive statistics of chemical indexes analyzed at extract 2

Lot	Analize	n	$\bar{x} \pm s_{\bar{x}}$	s	s^2	V%	min	max
E1	T. polyphenols	5	23,56 ± 0,32	0,73	0,528	3,08	22,60	24,20
	Flavons	3	5,92 ± 0,03	0,06	0,0032	0,96	5,85	6,00
	Flavonoids t.	3	16,52 ± 1,04	2,33	5,422	14,10	13,10	19,00
	A.antioxidantă	4	24,45 ± 0,66	1,31	1,7211	5,37	23,22	26,23
E2	T. polyphenols	5	27,67 ± 0,24	0,42	0,173	1,50	27,20	28,00
	Flavons	3	5,65 ± 0,03	0,05	0,0025	0,88	5,60	5,70
	Flavonoids t.	3	16,90 ± 0,12	0,20	0,04	1,18	16,70	17,10
	Antioxidant act.	4	26,71 ± 0,65	1,13	1,2729	4,22	25,43	27,56
E3	T. polyphenols	5	26,87 ± 0,33	0,58	0,333	2,15	26,20	27,20
	Flavons	3	5,18 ± 0,02	0,03	0,0008	0,56	5,15	5,20
	Flavonoids t.	3	16,83 ± 0,19	0,32	0,1033	1,91	16,60	17,20
	Antioxidant act.	4	26,21 ± 0,68	1,18	1,3843	4,49	25,00	27,35
E4	T. polyphenols	5	26,80 ± 0,23	0,40	0,16	1,49	26,40	27,20
	Flavons	3	5,18 ± 0,02	0,03	0,0008	0,56	5,15	5,20
	Flavonoids t.	3	16,17 ± 0,27	0,46	0,2133	2,86	15,90	16,70
	Antioxidant act.	4	26,50 ± 1,25	2,17	4,6926	8,18	24,15	28,42
E5	T. polyphenols	5	27,60 ± 0,46	0,80	0,64	2,90	26,80	28,40
	Flavons	3	5,38 ± 0,04	0,08	0,0056	1,40	5,30	5,45
	Flavonoids t.	3	18,05 ± 0,14	0,25	0,0625	1,39	17,80	18,30
	Antioxidant act.	4	19,90 ± 1,09	1,88	3,5344	9,45	18,02	21,78
E6	T. polyphenols	5	26,80 ± 0,35	0,60	0,36	2,24	26,20	27,40
	Flavons	3	4,95 ± 0,01	0,03	0,0006	0,51	4,95	5,00
	Flavonoids t.	3	19,50 ± 0,17	0,30	0,09	1,54	19,20	19,80
	Antioxidant act.	4	20,59 ± 0,46	0,80	0,632	3,86	19,80	21,39
E7	T. polyphenols	5	26,10 ± 0,98	1,70	2,89	6,51	24,40	27,80
	Flavons	3	5,00 ± 0,03	0,05	0,0025	1,00	4,95	5,05
	Flavonoids t.	3	19,15 ± 0,14	0,25	0,0625	1,31	18,90	19,40
	Antioxidant act.	4	24,26 ± 1,43	2,48	6,1256	10,20	21,78	26,73

CONCLUSIONS

The amount of total polyphenols after two weeks was higher than detected in the extract of fresh pollen, showing an upward dynamic of these compounds in the presence of methanol. Values were maximum after two weeks (extract 1) were increased after the first week, remaining in close level after the second week (extract 2). The amount of flavanols detected followed a downward trajectory, registering a decrease of mean values, both after first and second week (extract 2). In extract 1, the values obtained were close for both the first and second week after for all experimental conditions.

Quantities of total flavonoids, increased after both the first and the second week. The maximum level of flavonoids was recorded after the second week (extract 2), and between the first and second week in extract 1. The antioxidant activity detected in the extracts was lower in the two weeks kept extracts than in fresh ones. Maximum anti radical activity was greatest in fresh pollen extract (extract 1), reaching a peak value after the first week in extract 2.

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