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THE DYNAMICS OF TOTAL POLYPHENOLS, FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF BEEPOLLEN COLLECTED FROM MOLDAVIA AREA, ROMANIA

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ABSTRACT. The analysis sought to study the determination in timp and different conditions (temperature and light) of total polyphenols, flavones, total flavonoids and antioxidant activity from beepollen obtained with pollen colectors from Moldavia, Romania. Two methods vave been used, consisting in different concentrations of the methanolic solvent used. Analyses were performed on fresh pollen, stored for one and two weeks; at 4°C and 22°C; presence or absence of natural light. For every determination, five repetitions were made. There were fluctuations in the amounts of compounds, depending on the storage conditions and on the solvent used. The total polyphenols quantity ranged from 24.73 mg GAE/g to 28.8 mg GAE/g, when the methanolic solvent concentration was 96 % and from 21.67 mg GAE/g to 26.50 mg GAE/g, when the methanolic solvent concentration was 70 %. The flavones detected ranged from 7.23 mg QE/g to 8.93 mg QE/g (methanolic solvent 96%), and from 5.45 mg QE/g to 6.35 mg QE/g (methanolic solvent 70%). Total flavonoids have recorded values between 12.6 mg QE/g and 20.44 mg QE/g for the m.s. of 96%, and between 14.6 mg QE/g and 17.47 mg QE/g for the m.s. of 70%. The detected antiradical activity ranged from 22.68% to 29.78% inhibition (m.s. 96%), respectively from 21.31% to 25.55 % inhibition (m.s. 70%).

Key words: Antioxidant activity; Dynamics; Flavonoids; Pollen; Polyphenols.

REZUMAT – Dinamica polifenolilor totali, a flavonoidelor şi a activităţii antioxidante a polenului apicol din zona Moldovei. Analizele efectuate au urmărit determinarea, în timp şi în diferite condiţii de temperatură şi lumină, a cantităţii de polifenoli totali, flavone, flavonoide totale, precum şi a activităţii antioxidante a polenului poliflor, obţinut cu ajutorul colectoarelor din zona Moldovei. S-au utilizat doua metode, constând în concentraţii diferite ale solventului metanolic, utilizat la extracţie. Analizele au fost efectuate pe polen proaspăt, polen păstrat o săptămână şi polen păstrat două săptămâni, la 4°C şi 20°C, în prezenţa sau în absenţa luminii naturale. Pentru fiecare

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determinare s-au efectuat câte cinci repetiţii. S-au detectat fluctuaţii în cantităţile de compuşi urmăriţi, atât în funcţie de condiţiile de păstrare, cât şi în funcţie de solventul utilizat. Cantitatea de polifenoli totali a variat între 24,73 mg GAE/g şi 28,8 mg GAE/g, atunci când solventul metanolic utilizat a avut o concentraţie de 96%, respectiv de 21,67 mg GAE/g şi de 26,50 mg GAE/g, când solventul metanolic a fost de 70%. Cantităţile de flavone detectate au fost cuprinse între 7,23 mg QE/g şi 8,93 mg QE/g, pentru solvent metanolic 96%, respectiv între 5,45 mg QE/g şi 6,35 mg QE/g, pentru solvent cu o concentaţie de 70%. Flavonoidele totale au avut valori detectate cuprinse între 12,6 mg QE/g şi 20,44 mg QE/g (s.m. 96%), respectiv 14,6 mg QE/g şi 17,47 mg QE/g (s.m. 70%). Pentru activitatea antioxidantă s-au detectat valori cuprinse între 22,68% şi 29,78% inhibiţie (s.m. 96%), respectiv 21,31% şi 25,55% inhibiţie (s.m. 70%).

Cuvinte cheie: activitate antioxidantă; dinamică; flavonoide; polen; polifenoli.

INTRODUCTION

Beepollen is an excellent remedy in various health problems, while representing a balanced and nutritious food. Biological composition of pollen varies from one species to another. In the same time, its quality is dependent on a series of factors like: conditioning, packing and storage. Different studies show that the polyphenols present in beepollen (flavonoids, fenolic acids) largely determines its antioxidant activity (Gheldof *et al*., 2002; Berreta *et al.,* 2005; Pietta, 2000). Numerous studies have demonstrated a large number of medicinal herbs, as well as fruit and leaves of shrubs, synthesize phytochemicals with potential antioxidant activity, that can be used as a natural source of compounds to stop the propagation of free radicals. Antioxidants in foods such as vitamin C, vitamin E, carotenoids, have generated particular interest as "Stoppers" of degenerative diseases. There are studies indicating that polyphenols such as phenolic acids and flavonoids, are antioxidants considered to be stronger than vitamin C and vitamin E; other studies have shown a good correlation between antioxidant activity of some plants (Aljadi and Kamaruddin, 2004) and their content in polyphenols (Cocan *et al.,* 2006).

Characterisation of the amount of polyphenols and other components of beepollen can be responsable for the antioxidant effects, is essential to enrich our knoledge about pollen as an important source of antioxidants. Some studies have begun to appear on the pollen content of bioactive elements (Mărghitaş *et al.,* 2009; Prelipcean *et al.,* 2011) but data on the quantitative dynamics of polyphenols in different conditions are practically nonexistant so far. Today there is an increased demand for organic products in human diets and with antioxidant properties. The lattest are important in combating the negative effects of various synthetic additives, with adverse effects on the health.

This study sought to reveal the content in total polyphenols, flavones and total flavonoids correlated with antioxidant activity, of a known

poliflor pollen mix in different conditions, in order to obtain an image on the dynamics of this bee product.

MATERIALS AND METHODS

The poliflor pollen used was collected with pollen colectors, in a apiary from Iaşi, Romania. Samples of fresh pollen (directly from plant species) were also collected from the range of the bee yard. The determination of the vegetal species of pollen was made microscopicaly, by comparison with the freshly collected pollen and with the determinators (Chifu *et al.,* 2002; Tarnavschi *et al.,* 1990)

To determine the total content of polyphenols, Folin-Ciocâlteu method was used (Folin *et al.,* 1927), taken over and modified by various authors (Ferreres *et al.,* 1996; Gheldof *et al.,* 2002), adapted for all types of matrices for the determination of total polyphenol content (Kim *et al.,* 2003; Singleton *et al.,* 1999). This method uses the spectrophotometric method that measures the absorbance at 760 nm after the addition of a specific reagent: fosfomolibdenic-fosfotungstic acid (Folin-Ciocâlteu, 1927).

In order to determine the quantities of polyphenols in pollen samples, a calibration curve was performed, with known concentrations of gallic acid (Meda et *al.,* 2005). The Folin-Ciocâlteu method, previously tested (Prelipcean and Bobiş, 2011), to determine polyphenols in pollen, is summarized in the following steps: 5 g of pollen were dissolved in 50 ml methanol 96% (method 1) and 70% methanol (method 2); as the concentration of 10% pollen was found to be highly concentrated, dilutions of extracts were made, to the concentration of 1%; mixtures were agitated with a ultraturax for 10 minutes and then ultrasonicated for 30 minutes; the resulting solutions were centrifuged for 20 minutes at 3500 rpm; 0.5 ml of supernatant was mixed with 2.5 ml of 0.2 N Folin-Ciocâlteu reagent for 5 minutes, then 2 ml of 75 g/l sodium carbonate solution was added; samples were incubated at room temperature and darkness for 2 hours, and absorbance was read at the spectrophotometer at 760 nm against a blank: Folin-Ciocâlteu + methanol + sodium carbonate. Results were expressed as mg GAE/g pollen (mg gallic acid equivalents per gram pollen).

Analysis of flavonols and total flavonoids was reported by different authors that have used different methods with specific reagents. The Dowd method (Zhishen *et al.,* 1999), taken over and modified by various authors (Arvouet-Grand *et al.,* 1994; Meda *et al.,* 2005; Kim *et al.,* 2003), is using a solution of aluminium chloride for a specific reaction (reacts with flavonoids present in the sample), giving a yellow color, the intensity of which being able to be determined spectrophotometrically at 415 nm. Using this method, small amounts of total flavonoids were reported in comparison with the total contentof polyphenols. This is because $AlCP_3$ only reacts with flavones and flavonols (Arvouet-Grand *et al.,* 1994). Extraction of flavonols: from the 1% pollen extract, 2 ml were taken (equivalent to 0.02 g pollen) and were mixed with a equal quantity of a solution of 2% aluminium chloride in methanol. After 10 minutes, the absorbance was read at the spectrophotometer at 415 nm. Extraction of total flavonoids: 1 ml of 1% pollen extract (equivalent of 0. 01g pollen) was mixed with 0.3 ml of 5% $NaNO_2$. After 5 minutes, 0.3 ml of $AlCP_3$ 10% were added. Samples were agitated, and after 6 minutes were neutralized with 2 ml of NaOH 1M. Absorbance reading was

performed using the calibration curve. Results were expressed as the average of the three repetitions in mg quercetin equivalents per g pollen (mg QE/g pollen).

The method used by some authors (Benzie and Strain, 1999; Chen *et al.,* 2000) for measuring the antioxidant activity, was adapted (Velazquez *et al.,* 2003) after the original method of Brand-Williams (Brand-Williams *et al.,* 1995), and used in this study. This method (DPPH) is based on the measuring the ability of antioxidants to block the 2,2-difenil-1-picril-hidrazil radical. The DPPH free radical, colored in dark blue, is reduced to hydrazine, being descoloured when reacts with different hydrogen donors. This ability is evaluated using the ultraviolet spectrophotometry, on the basis that the signal intensity after reaction is inversely proportional with the antioxidant concentration and with the reaction time, the change being seen at 517 nm. The inhibition percentage (IP) was calculated whit the formula: $IP(\%\,\text{inhibition}) = \left(A0 - \frac{PA}{A0}\right) \times 100$, where A0 is the absorbance of blank solution, and PA is the absorbance of the sample after 15 minutes. For samples preparation two extraction solvents were used: methanol 96% and methanol 70%. These were mixed with 2.5 ml DPPH solution of 0.03 mg/ml concentration (in methanol). This was followed by a phase of mixing and incubation at room temperature and no light, for 15 minutes, after which, the DPPH absorbance of the solution left unconsumed was determined at 517 nm compared to the blank.

The experimental plots were organised as follows: P1 – fresh pollen; P2 – pollen preserved 7 days, at 4°C and no light; P3 – pollen preserved 7 days, at 22°C and natural light; P4 – pollen preserved 7 days, at 22°C and no light; P5 – pollen preserved 14 days, at 4°C and no light; P6 – pollen preserved 14 days, at 22°C and natural light; P7 – pollen preserved 14 days, at 22°C and no light.

RESULTS AND DISCUSSION

The origin of the pollen used was microscopicaly determined, by comparison with the fresh pollen gathered from determined species in the same area as the beepollen, and also by comparison with data from palynological literature (Tarnavschi *et al.,* 1990). We found pollen from following species: *Robinia pseudoacacia* L., *Brassica napus* L., *Papaver hybridum* L., *Onobrychis viciifolia* Scop., *Phacelia tanacetifolia* Benth., *Centaurea cyanus* L., *Trifolium hybridum* L., *Salvia sp*., *Taraxacum officinale* Cass..

The quantity of polyphenols recorded values of 27.64 mg GAE/g pollen*; 23.56 mg GAE/g pollen** for the fresh pollen.Using method 1* (96% methanolic solvent), after 7 days, there was a decrease in the value detected for the pollen stored at 4°C, in the absence of the light (24.73 mg GAE/g pollen), followed by an increase of value, after 14 days (27.4 mg GAE/g pollen) *(Figure 1).* The values detected for the lots held at 22°C, did not manifested significant valoric fluctuations after 7 days (P3 27.47 mg GAE/g pollen; P4 27.8 mg GAE/g pollen). After 14 days, the detected values have increased for P6, to a average of 28.8 mg GAE/g pollen. For P7, a average value of 27.1 mg GAE/g pollen was detected *(Table 1).*

Table 1 - Descriptive statistics of chemical indicators analysed by method 1

Lot	Analises	n	$\bar{x} \pm s_{\bar{x}}$	s	s^2	V%	min	max
L1 Fresh pollen	**T.polyphenols** (mg GAE/g pollen)	5	27.64 ± 0.35	0.79	0.628	2.87	26.40	28.40
	Flavones (mg QE/g pollen)	3	8.57 ± 0.31	0.69	0.482	8.10	8.00	9.35
	T.flavonoides (mg QE/g pollen)	3	20.44 ± 2.72	6.08	36.98	29.75	10.80	25.20
	Antioxidant activity (% inhibition)	4	24.18 ± 0.51	1.01	1.021	4.18	23.22	25.41
L2 7 days; 4˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	24.73 ± 0.64	1.10	1.213	4.45	24.00	26.00
	Flavones (mg QE/g pollen)	3	7.23 ± 0.07	0.13	0.015	1.74	7.10	7.35
	T.flavonoides (mg QE/g pollen)	3	16.73 ± 1.11	1.91	3.663	11.44	14.70	18.50
	Antioxidant activity (% inhibition)	4	23.37 ± 0.69	1.19	1.417	5.09	22.02	24.27
L3 7 days; 22˚C; hv	**T.polyphenols** (mg GAE/g pollen)	5	27.47 ± 0.84	1.45	2.093	5.27	25.80	28.40
	Flavones (mg QE/g pollen)	3	7.28 ± 0.10	0.18	0.0308	2.41	7.10	7.45
	T.flavonoides (mg QE/g pollen)	3	14.07 ± 1.90	3.29	10.803	23.37	11.30	17.70
	Antioxidant activity (% inhibition)	4	25.24 ± 0.52	0.91	0.826	3.60	24.27	26.07
L4 7 days; 22˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	27.80 ± 0.81	1.40	1.96	5.04	26.20	28.80
	Flavones (mg QE/g pollen)	3	8.08 ± 0.04	0.08	0.0058	0.94	8.00	8.15
	T.flavonoides (mg QE/g pollen)	3	16.17 ± 1.18	2.05	4.203	12.68	13.80	17.40
	Antioxidant activity (% inhibition)	4	26.07 ± 1.78	3.09	9.5546	11.86	23.37	29.44
L5 14 days; 4˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	27.40 ± 0.46	0.80	0.64	2.92	26.60	28.20
	Flavones (mg QE/g pollen)	3	8.35 ± 0.03	0.05	0.0025	0.60	8.30	8.40
	T.flavonoides (mg QE/g pollen)	3	11.87 ± 0.66	1.15	1.3233	9.69	10.70	13.00
	Antioxidant activity (% inhibition)	4	22.68 ± 1.58	2.73	7.452	12.04	19.95	25.41
L6 14 days; 22˚C; hv	**T.polyphenols** (mg GAE/g pollen)	5	28.80 ± 0.81	1.40	1.96	4.86	27.40	30.20
	Flavones (mg QE/g pollen)	3	8.93 ± 0.04	0.08	0.0056	0.84	8.85	9.00
	T.flavonoides (mg QE/g pollen)	3	12.80 ± 0.23	0.40	0.16	3.13	12.40	13.20
	Antioxidant activity (% inhibition)	4	29.78 ± 2.73	4.10	16.81	13.77	25.68	33.88
L7 14 days; 22˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	27.10 ± 0.29	0.50	0.25	1.85	26.60	27.60
	Flavones (mg QE/g pollen)	3	8.78 ± 0.07	0.13	0.0156	1.42	8.65	8.90
	T.flavonoides (mg QE/g pollen)	3	12.60 ± 0.35	0.60	0.36	4.76	12.00	13.20
	Antioxidant activity (% inhibition)	4	27.46 ± 1.34	2.32	5.382	8.45	25.14	29.78

Table 2 - Descriptive statistics of chemical indicators analysed by method 2

Lot	Analises	n	$\bar{x} \pm s_{\bar{x}}$	s	s^2	V%	min	max
L1 Fresh pollen	**T.polyphenols** (mg GAE/g pollen)	5	23.56 ± 0.32	0.73	0.528	3.08	22.60	24.20
	Flavones (mg QE/g pollen)	3	5.92 ± 0.03	0.06	0.0032	0.96	5.85	6.00
	T.flavonoides (mg QE/g pollen)	3	16.52 ± 1.04	2.33	5.422	14.10	13.10	19.00
	Antioxidant activity (% inhibition)	4	24.45 ± 0.66	1.31	1.7211	5.37	23.22	26.23
L2 7 days; 4˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	24.07 ± 0.33	0.58	0.3333	2.40	23.40	24.40
	Flavones (mg QE/g pollen)	3	5.90 ± 0.03	0.05	0.0025	0.85	5.58	5.95
	T.flavonoides (mg QE/g pollen)	3	15.50 ± 0.42	0.72	0.52	4.65	14.70	16.10
	Antioxidant activity (% inhibition)	4	23.67 ± 2.29	3.96	15.69	16.73	20.00	27.87
L3 7 days; 22˚C; hv	**T.polyphenols** (mg GAE/g pollen)	5	21.67 ± 0.57	0.99	0.973	4.55	21.00	22.80
	Flavones (mg QE/g pollen)	3	5.45 ± 0.03	0.05	0.0025	0.92	5.40	5.50
	T.flavonoides (mg QE/g pollen)	3	17.10 ± 1.30	2.25	5.07	13.17	14.90	19.40
	Antioxidant activity (% inhibition)	4	24.34 ± 1.30	2.26	5.109	9.29	22.25	26.74
L4 7 days; 22˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	22.87 ± 0.59	1.03	1.053	4.49	22.00	24.00
	Flavones (mg QE/g pollen)	3	6.05 ± 0.13	0.22	0.0475	3.60	5.90	6.30
	T.flavonoides (mg QE/g pollen)	3	17.00 ± 0.40	0.70	0.49	4.12	16.20	17.50
	Antioxidant activity (% inhibition)	4	23.37 ± 0.47	0.81	0.0653	3.46	22.70	24.27
L5 14 days; 4˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	26.50 ± 0.40	0.70	0.49	2.64	25.80	27.20
	Flavones (mg QE/g pollen)	3	6.35 ± 0.12	0.20	0.04	3.15	6.15	6.55
	T.flavonoides (mg QE/g pollen)	3	17.47 ± 0.55	0.95	0.9033	5.44	16.50	18.40
	Antioxidant activity (% inhibition)	4	25.55 ± 1.50	2.60	6.734	10.16	22.95	28.14
L6 14 days; 22˚C; hv	**T.polyphenols** (mg GAE/g pollen)	5	24.90 ± 0.40	0.70	0.49	2.81	24.20	25.60
	Flavones (mg QE/g pollen)	3	6.28 ± 0.04	0.08	0.0056	1.20	6.20	6.35
	T.flavonoides (mg QE/g pollen)	3	14.60 ± 0.17	0.30	0.09	2.05	14.30	14.90
	Antioxidant activity (% inhibition)	4	25.41 ± 0.63	1.09	1.188	4.29	24.32	26.50
L7 14 days; 22˚C; -hv	**T.polyphenols** (mg GAE/g pollen)	5	24.10 ± 0.06	0.10	0.01	0.41	24.00	24.20
	Flavones (mg QE/g pollen)	3	5.65 ± 0.03	0.05	0.0025	0.88	5.60	5.70
	T.flavonoides (mg QE/g pollen)	3	15.27 ± 0.66	1.15	1.3233	7.54	14.10	16.40
	Antioxidant activity (% inhibition)	4	21.31 ± 0.31	0.55	0.297	2.56	20.77	21.86

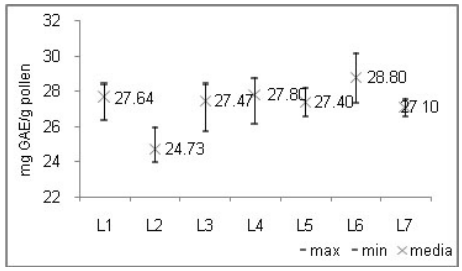

Figure 1- Dynamics of total polyphenols, method 1

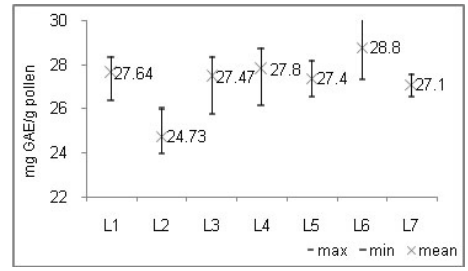

Figure 2 - Dynamics of total polyphenols, method 2

Using method 2** (70% methanolic solvent), the polyphenols detected had a ascendent trend for the pollen stored at 4°C, in the absence of the light (P2 24.07 mg GAE/g pollen; P5 26.5 mg GAE/g pollen) *(Figure 2).* The lots held at 22°C, have manifested a slight decrease after the first week (P3 216.7 mg GAE/g pollen; P4 22.,87 mg GAE/g pollen), followed by an increase after the second week (P6 24.9 mg GAE/g pollen; P7 24.1 mg GAE/g pollen) *(Table 2).*

Overall, the polyphenolics dynamics trend is more visible by method 2. With both methods, there were slight decreases in the mean values after the first week, indicating a possible fluctuating permeability of pollen cover, dependent on storage time, temperature and light.

The flavones registred values of 8.57 mg QE/g pollen*; 5.92 mg QE/g pollen** for the fresh pollen. Using method 1 (96% methanolic solvent), as in the total polyphenols case, the detected values for flavones, after 7 days, registred a slight decrease, recording increases in value for all experimental groups after the second week *(Figure 3).*

For the pollen stored at 4°C, the increase registred was from P2 7.23 mg QE/g pollen, to P5 8.35 mg QE/g pollen. Values increases were noticed in the case of lots held at a temperature of 22°C. P3 7.28 mg QE/g pollen; P6 8.93 mg QE/g pollen and P4 8.08 mg QE/g pollen; P7 8.78 mg QE/g pollen) *(Table 1).*

By method 2 (70% methanolic solvent), the valoric fluctuations were less conclusive. Thus, the amount of flavons for the pollen kept at 4°C had increased from P2 5.9 mg QE/g pollen, after 7 days, to P5 6.35 mg QE/g pollen, after 14 days *(Figure 4).* The detected values for the pollen kept at 22°C, showed different dynamics of the two group, depending on the light. Thus, the lot stored at natural light, registered an increase from P3 5.45 mg QE/g pollen, to P6 6.28 mg QE/g pollen. The lot stored in darkness, had a value of P4 6.05 QE/g pollen after 7 days, followed by P7 5.65 mg QE/g pollen, after 14 days *(Table 2).*

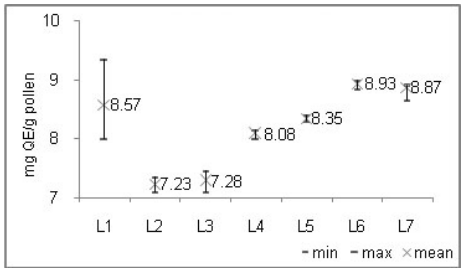

Figure 3 - The dynamics of flavons, method 1

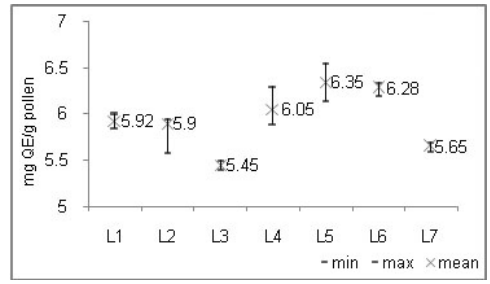

Figure 4 - The dynamics of flavons, method 2

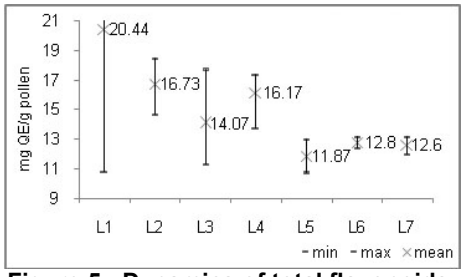

Figure 5 - Dynamics of total flavonoids, method 1

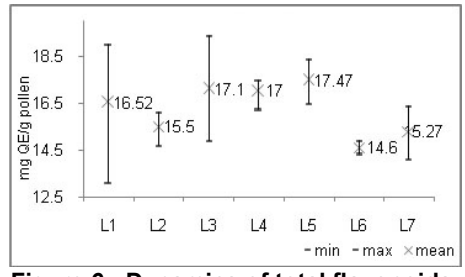

Figure 6 - Dynamics of total flavonoids, method 2

Both methods have revealed a dynamic generally upward of the flavons, clearer and more visible by method 1.

The quantity of total flavonoids registered values of 20.44 mg QE/g pollen*; 16.52 mg QE/g pollen** for the fresh pollen. By method 1, the total flavonoids presented a descendent dynamic, for all the storage conditions, after 7 and 14 days. Thus, for the pollen stored at 4°C, the values decreased from P2 16.73 mg QE/g pollen, after 7 days, to 11.87 mg QE/g pollen after 14 days *(Figure 5).* The same evolution was evidenced in the lots held at 22°C: P3 14.07 mg QE/g pollen; P6 12.8 mg QE/g pollen and P4 16.17 mg QE/g pollen; P7 12.6 mg QE/g pollen *(Table 1).*

By method 2, the total flavonoids dynamics presented a ascendent evolution in the case of pollen kept at 4°C: P2 15.5 mg QE/g pollen after 7 days, followed by P5 17.47 mg QE/g pollen, after 14 days *(Figure 6).* For the pollen kept at 22°C, the values increased after the first week, followed by a decrease after the second week: P3 17.1 mg QE/g pollen; P6 14.6 mg QE/g pollen and P4 17 mg QE/g pollen; P7 15.27 mg QE/g pollen *(Table 2).*

Method 1 revealed a decrease of the total flavonoids, and by method 2, the decrease was visible only for the lots kept at 22°C.

The antioxidant activity (by DPPH), also used two different

concentrations of the methanolic solvent. The IC_{50} value, representing the probe amount concentration that inhibit the DPPH radical in a 50% percent, was obtained by using different concentrations of pollinic extract (0.1 – 0.25%). The value was IC_{50}=0.211 for the fresh pollen. The values were 24.18% inhibition at a methanolic solvent concentration of 96% and 24.45% inhibition, for a methanolic solvent of 70%.

Using method 1, the detected values were in a slight decrease for the pollen stored at 4°C (P2 23.37% inhibition; 22.68% inhibition). In the case of the pollen kept at 22°C, the inhibition values showed increases: P3 25.24% inhibition; 29.78% inhibition and 26.07% inhibition; 27.46% inhibition *(Table 1) (Figure 7).* By method 2, the dynamics slightly decreased after 7 days, increasing after 14 days at P5 and P6 *(Table 2) (Figure 8).*

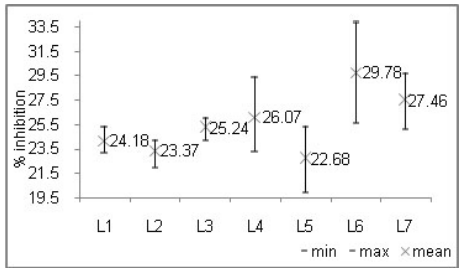

Figure 7 - Dynamics of antioxidant activity, method 1

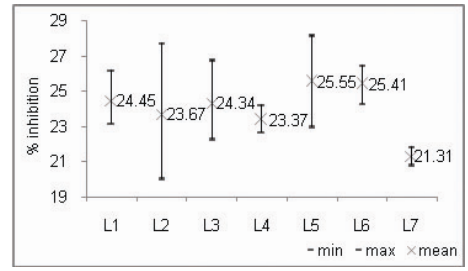

Figure 8 - Dynamics of antioxidant activity, method 2

CONCLUSIONS

The quantity of total polyphenols registered a slight decrease after the first week (P2, P3), followed by an slight increase after the second week (P5, P6), using a extraction methanolic solvent of 96 % (method 1). Using method 2, the dynamics was ascendent, the differences being more visible after the second week (P5, P6, P7).

The dynamics of total polyphenols was an ascendent one, being more visible by the second method (70 % methanolic solvent). The fluctuating dynamic of the total polyphenols quantity, depends on the storing conditions and on the methanolic solvent concentration used. However, the increasing values may be explained by the disintegration in time of the pollen coating and release of increasing amounts of polyphenols. The higher quantity of total polyphenols, after two weeks, shows that they are gradually released. Thus, the biological action of these compounds can be highlighted better in pollen preserved at least two weeks.

The flavones values dynamics, had a descendent evolution after the first week, being followed by an increase of the detected quantities

after the second week (method 1). Using the second method, the situation was similar, except the pollen stored at room temperature (22°C) and no light. In this case, there was an increase of the values after the first week and a slight decrease after the second week. The fluctuating dynamic can be also explained by the pollen coatings desintegration and the gradually release of the compounds. Thus, the specifical biological properties can be better observed starting with the second storage week.

The total flavonoids dynamics, manifested a decreasing values trend for all the storage conditions (using a methanolic solvent of 96 %). With a methanolic solvent of 70%, the average values of the detected total flavonoids showed fluctuations. At this point, the differences were visible, depending on the storage conditions. From the pollen stored at 4°C in the absence of the light, the highest quantity of total flavonoids was obtained. The biological activity of these compounds can be best evidenced after a storage period of one week.

The antioxidant activity showed fluctuations in the average values, depending on the storage conditions. The dynamics had a descendent evolution for the pollen stored at 4°C, in the absence of the light and a ascendent one, for the pollen kept at room temperature (22°C) (method 1,96 % methanolic solvent). Using the second method (70% methanolic solvent), the antioxidant activity decreased after the first week for all the storage conditions. This trend also continued for the pollen kept at 22°C and no light, after the second week. For the other conditions, the values registered increases. In the determination of antioxidant activity, an essential role is played by the concentration of the (methanolic) solvent used, by its action on the pollinic wall, releasing with a higher or lesser efficiency the active principles contained in pollen. By analysing the antioxidant activity dynamics, we emphasized that this is more visible after the second week. Further research is needed to observe the evolution of compounds in pollen for longer periods.

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