DYNAMICS OF SOME BIOCHEMICAL PROPERTIES OF BEE POLLEN STORED IN DIFFERENT CONDITIONS

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Abstract

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. At the same time, the quality is dependent on several factors such as pollen harvesting period, packaging and storage methods, conditioning. Our research aims to follow the dynamic changes in the biochemical composition, occurring in bee pollen, in different storage conditions. Working methods used were the bee pollen harvesting by collectors of pollen, his conditioning and keeping it in different conditions (temperature and light) for 3 and 6 months. Later, chemical analysis were made, aiming for each batch, the evolution of moisture, dry substance, minerals, fat, protein and easily hydrolyzable nitrogen. The data were analyzed by applying descriptive statistics and analysis of variance using unifactorial ANOVA test. The research undertaken complete the poor information on the dynamics of biochemical properties of pollen, looking to point some aspects on the storage conditions of this bee product.

Key words*:* pollen, dynamics, biochemical properties, storage conditions

INTRODUCTION

Pollen collected by bees is a food with a high biological value. It is also the only natural protein source for bees. In beekeeping, pollen is important as an economic factor in additional revenue and is an essential product in the life and optimal development of the bee colony.

Even if bee pollen nutritional properties have attracted public attention, its effects in human nutrition and bees nutrition are only partially understood. There are numerous researches carried out on pollen content, made by various authors [1]; [2]; [3]; [7]; [9]; [12]; [13]; [14], but studies on the dynamics of chemical properties are very few [11]. However, many tests are performed on pollen with geografical [10] and botanical specification [4], less on pollen mixes which is the main way of pollen marketing in Romania. Primary factors influencing the quality of bee pollen are the methods of preparation and storage. According to the biochemical composition and conditioning of bee pollen, quality is directly affected.

MATERIAL AND METHODS

Biological samples, consisting of bee polifit pollen, were collected in the apiary of Faculty of Animal Husbandry, in a pastoral area in the vicinity of forest Bârnova. Collection period was in May (2009) in preparation for harvesting the lime. The amount of pollen collected and used for analysis was approximately 3 kg.

Pollen was homogenized and divided into lots, which were later kept in different conditions (fig. 1).

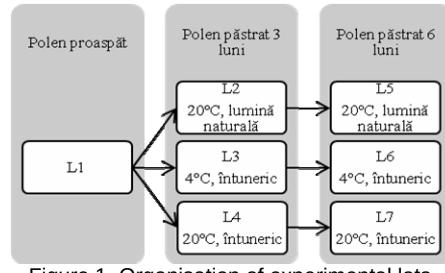

Figure 1. Organisation of experimental lots

Samples were held in glass containers provided with a screw cap. Consignments which required the absence of light were wrapped in foil wrap. Room and refirgerating temperature was monitored daily. The mean temperature was ≈ 20°C for the room environment and 4°C for refrigeration conditions. In his conditioning, fresh pollen was brought to an average humidity of 9.19%

recommended in the literature conformity [8]. To assess the amount of water and dry substance, the oven drying method was used. The amount of minerals was determined by the calcination method (SR ISO 936:1998).

Brute fat was determined by Soxhlet method, direct version, with a extraction device. (SR ISO 1443:2008). To determine brute protein, Kjeldahl Velp method was used. (SR ISO 937:2007). Determination of easily hydrolyzable nitrogen (ammonia) was carried out by distillation and abstraction in sulfuric acid followed by titration [5].

The raw data were analyzed for each batch, using descriptive statistics (arithmetic mean, standard deviation, variance, standard error of the mean, index of variability), and by analysis of variance of means by applying ANOVA unifactorial.

RESULTS AND DISCUSSION

The data obtained were organized in tables according to each parameter. Absolute humidity had the basic value, an average of 9.19% for L1 (fig.2). After 3 months of storage, arithmetic averages showed insignificant decreases in value (table 1a) at L2 and L3 and a very significant increase (table 2a) at L4.

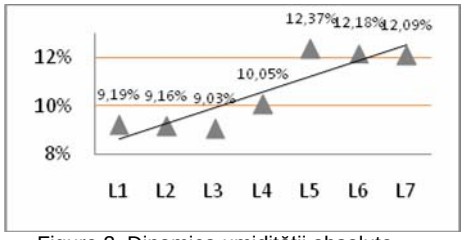

Figure 2. Dinamica umidității absolute

After six months, tests have shown increases in value for all storage conditions, detecting increasing values of 30% from baseline (L5, L6, L7), showing very significant differences (table.3).

For dry matter, values had a downward dynamic (table 1a), value based on 90.73% for L1 (fig.3). After three months, the amount of dry matter calculated had a value decrease to 89.92% for L2, 89,43% for L3 and 89,93% for L4. Downward trajectory was preserved after 6 months for all experimental conditions, being recorded values of 87,18% for L5, 87,9% for L6 and 87,95% for L7. L1 differences from all lots were very significant (table 2a).

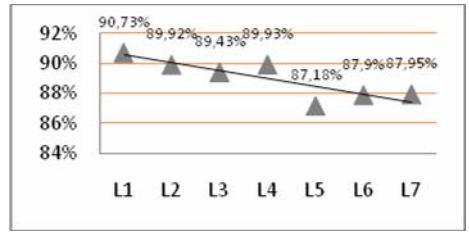

Figure 3. Dynamic of dry substance

The amount of mineral substances detected had an average of 2.83% for fresh pollen L1 (table 1a and fig. 4). Dynamics showed a downward curve after 3 months for all storage conditions, being most pronounced in sample L4 with an average of 2.48%. The downward trend continued after 6 months, lowest values were recorded in groups L5 and L7, with averages of 2.24%. Differences between groups were mostly insignificant (table 2a).

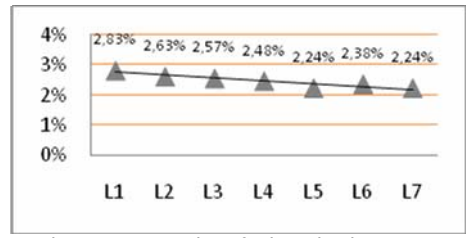

Figure 4. Dynamics of mineral substances

Calculated amount of organic substances, has also been declining over the period of storage. Highest average was recorded for fresh pollen L1, of 87.98% (table 1a and fig. 5). After three months, the calculated values showed lower values: 87.24% for L2, 86.86% for L3 and 87.23% for L4. After six months, the pace continued downward, reaching a minimum value in sample L5, of 84.93%. Very significant differences from L1 were recorded after 6 months (table 2a).

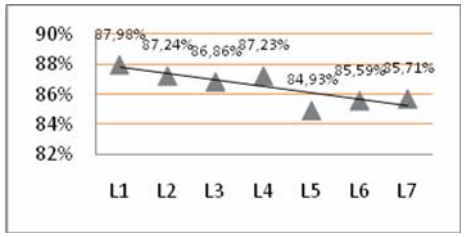

Figure 5. Dynamics of organic substance

Table 1a. Descriptive statistics indicators

Absolute humidity (%)	n	Calculated statistical indicators:					Limits of variation		% to L1
		$\bar{X} \pm$	s $\bar{X}$	s	s^2	V %	minimum	maximum	
L1	4	9,19	0,07	0,15	0,021	1,60%	8,99	9,33	100%
L2	4	9,16	0,05	0,09	0,009	1,1%	9,04	9,26	99,67%
L3	4	9,03	0,11	0,22	0,046	2,4%	8,73	9,24	98,25%
L4	4	10,05	0,04	0,08	0,005	0,74%	9,96	10,13	109,35%
L5	4	12,37	0,04	0,09	0,008	0,7%	12,28	12,48	134,60%
L6	4	12,18	0,08	0,17	0,029	1,4%	11,96	12,35	132,53%
L7	4	12,09	0,05	0,11	0,013	1,0%	11,95	12,23	131,55%
Dry substance (%)									
L1	4	90,73	0,14	0,29	0,082	0,32%	90,33	91,01	100%
L2	4	89,92	0,04	0,09	0,007	0,1%	89,85	90,05	99,10%
L3	4	89,43	0,11	0,21	0,040	0,2%	89,19	89,68	98,56%
L4	4	89,93	0,04	0,07	0,005	0,08%	89,87	90,04	99,11%
L5	4	87,18	0,24	0,48	0,237	0,6%	86,68	87,62	96,08%
L6	4	87,90	0,05	0,11	0,013	0,1%	87,79	88,04	96,88%
L7	4	87,95	0,03	0,07	0,005	0,1%	87,86	88,04	96,93%
Mineral substances (%)									
L1	4	2,83	0,28	0,57	0,321	20,03%	2,53	3,68	100%
L2	4	2,63	0,23	0,47	0,223	17,9%	2,12	3,25	92,93%
L3	4	2,57	0,17	0,35	0,122	13,6%	2,12	2,97	90,81%
L4	4	2,48	0,02	0,04	0,001	1,44%	2,44	2,52	87,63%
L5	4	2,24	0,04	0,09	0,008	4,1%	2,12	2,33	79,15%
L6	4	2,38	0,05	0,11	0,012	4,6%	2,28	2,53	84,09%
L7	4	2,24	0,01	0,02	0,001	1,0%	2,21	2,26	79,15%
Organic substances (%)									
L1	4	87,98	0,33	0,67	0,444	0,76%	86,99	88,44	100%
L2	4	87,24	0,23	0,47	0,225	0,5%	86,65	87,79	99,15%
L3	4	86,86	0,13	0,27	0,078	0,3%	86,47	87,11	98,72%
L4	4	87,23	0,25	0,51	0,263	0,58%	86,47	87,59	99,14%
L5	4	84,93	0,28	0,56	0,321	0,7%	84,35	85,46	96,53%
L6	4	85,59	0,08	0,17	0,028	0,2%	85,51	85,85	97,28%
L7	4	85,71	0,03	0,05	0,003	0,1%	85,65	85,78	97,41%
Fat (%)									
L1	4	5,902	0,05	0,11	0,011	1,847%	5,82	6,06	100%
L2	4	7,31	0,11	0,21	0,041	2,77%	7,11	7,59	123,85%
L3	4	7,48	0,05	0,11	0,011	1,4%	7,34	7,59	126,73%
L4	4	7,82	0,01	0,03	0,001	0,35%	7,80	7,86	132,49%
L5	4	6,32	0,02	0,04	0,002	0,8%	6,27	6,38	107,07%
L6	4	6,17	0,04	0,08	0,007	1,4%	6,09	6,29	104,54%
L7	4	6,66	0,03	0,05	0,003	0,9%	6,59	6,73	112,84%

Table 1b. Descriptive statistics indicators

Protein (%)	n	Calculated statistical indicators:					Limits of variation		% to L1
		$\overline{X} \pm$	$s_{\overline{X}}$	s	s^2	V %	minimum	maximum	
L1	4	24,56	0,17	0,35	0,12113	1,42%	24,12	24,97	100%
L2	4	22,79	0,35	0,69	0,4874	3,1%	22,03	23,68	92,73%
L3	4	23,39	0,38	0,76	0,5902	3,3%	22,44	24,18	95,23%
L4	4	22,52	0,48	0,96	0,93024	4,28%	21,59	23,87	91,69%
L5	4	24,78	0,07	0,15	0,02283	0,6%	24,6	24,93	100,89%
L6	4	23,79	0,02	0,05	0,00262	0,2%	23,74	23,86	96,86%
L7	4	23,5	0,06	0,13	0,01687	0,6%	23,34	23,62	95,68%
Easily hydrolyzable nitrogen (mg/100g)									
L1	4	2,08	0,06	0,11	0,0123	5,34%	2,01	2,24	100%
L2	4	2,08	0,06	0,12	0,01416	5,71%	2,01	2,26	100%
L3	4	2,69	0,24	0,48	0,23327	17,95	2,26	3,29	129,32%
L4	4	2,29	0,13	0,26	0,06737	11,36%	2,06	2,65	110,09%
L5	4	3,17	0,07	0,14	0,02072	4,5%	3,062	3,38	152,40%
L6	4	3,28	0,03	0,07	0,00534	2,2%	3,21	3,38	157,69%
L7	4	2,39	0,16	0,32	0,10831	13,8%	1,998	2,69	114,90%

Value found after determining the amount of fat was the lowest for L1: 5,902% (table 1a and fig. 5). After 3 months was an increase in the value averages for all storage conditions: L2 with 7.31%; L3 with 7.48%; L4 with 7.82%. After six months there has been a decrease in all means such: L5 with 6.32%; L6 with 6.17%; L7 with 6.66%. Differences between L1 and other groups were very significant (table 2a).

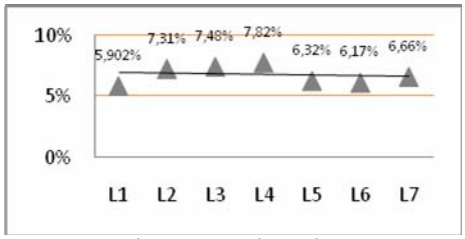

Figure 6. Fat dynamics

Protein dynamics values had a downward fluctuation after 3 months, calculating weak significant differences between L1 L3 and L1 L4 and distinct significant differences between L1 L2 (table 2a), followed by growth after 6 months (table 1b and fig.6). Averages found were 24.56% for L1, 22.79% for L2, 23.39% for L3; 22.52% for L4; 24.78% for L5; 23.79% for L6; 23.5% for L7. The most significant differences between groups were calculated for L5 L6 and L5 L7, highlighting very significant changes (table 2a) between groups kept under different conditions, after 6 months.

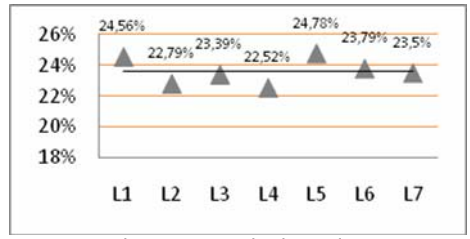

Figure 7. Protein dynamics

The dynamics of easily hydrolyzable nitrogen (ammonia), was an upward after three months and after six months, for all storage conditions (table 1a and fig. 7). Most significant increases after six months showed significant differences between L1 L5, L1 L7, temperature being the common factor behind this dynamic (table 2b).

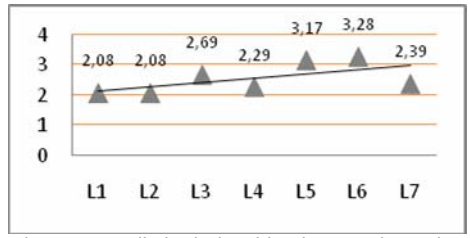

Figure 8. Easily hydrolyzable nitrogen dynamics

Also, most pronounced increase was recorded in groups L3 (3 months) and L6 (6 months), including pollen stored at 4° C in the dark. Significant differences were noticed between L2 L5 and L4 L7, kept under the same conditions (differing only time), where

the dynamic was very pronounced. For the analysis of variance, unifactorial ANOVA test was used, analyzing the differences between groups, both on the periods of storage (fresh pollen, pollen stored 3 months, pollen stored 6 months), and between storage conditions (temperature and light).

Table 2a. Analytical statistical indicators

Persued parameter	Lots to compare	F crit.	F_α	Level	Statistical significance	Persued parameter	Lots to compare	F crit.	F_α	Level	Statistical significance
Absolute humidity	L1 L2	0.09792	5.98737	0.05	**N.S.**	Dry substance	L1 L2	107.824	35.5075	0.001	***
	L1 L3	1.45722	5.98737	0.05	**N.S.**		L1 L3	123.165	35.5074	0.001	***
	L1 L4	111.104	35.5074	0.001	***		L1 L4	113.689	35.5074	0.001	***
	L1 L5	1355.06	35.5074	0.001	***		L1 L5	203.204	35.5074	0.001	***
	L1 L6	707.56	35.5074	0.001	***		L1 L6	978.468	35.5074	0.001	***
	L1 L7	962.739	35.5074	0.001	***		L1 L7	1197.21	35.5074	0.001	***
	L2 L3	1.21291	5.98737	0.05	**N.S.**		L2 L3	20.6554	13.7450	0.01	**
	L2 L4	111.104	35.5074	0.001	***		L2 L4	0.00774	5.98737	0.05	**N.S.**
	L2 L5	2344.70	35.5074	0.001	***		L2 L5	123.096	35.5074	0.001	***
	L3 L4	81.0691	35.5074	0.001	***		L3 L4	21.9058	13.7450	0.01	**
	L3 L6	525.927	35.5074	0.001	***		L3 L6	176.283	35.5074	0.001	***
	L4 L7	867.753	35.5074	0.001	***		L4 L7	1389.37	35.5074	0.001	***
	L5 L6	4.07047	5.98737	0.05	**N.S.**		L5 L6	8.27729	13.7450	0.01	*
	L5 L7	14.0490	13.7450	0.01	**		L5 L7	9.81540	13.7450	0.01	*
	L6 L7	0.6397	5.98737	0.05	**N.S.**		L6 L7	0.58878	5.98737	0.05	**N.S.**
Mineral substances (Ash)	L1 L2	0.27182	5.98737	0.05	**N.S.**	Organic matter	L1 L2	3.26893	5.98737	0.05	**N.S.**
	L1 L3	0.57400	5.98737	0.05	**N.S.**		L1 L3	9.5951	5.98737	0.05	*
	L1 L4	1.50831	5.98737	0.05	**N.S.**		L1 L4	3.14263	5.98737	0.05	**N.S.**
	L1 L5	4.11249	5.98737	0.05	**N.S.**		L1 L5	48.4031	35.5074	0.001	***
	L1 L6	2.40145	5.98737	0.05	**N.S.**		L1 L6	54.3638	35.5074	0.001	***
	L1 L7	4.28909	5.98737	0.05	**N.S.**		L1 L7	45.9110	35.5074	0.001	***
	L2 L3	0.04152	5.98737	0.05	**N.S.**		L2 L3	1.90459	5.98737	0.05	**N.S.**
	L2 L4	0.43409	5.98737	0.05	**N.S.**		L2 L4	0.00031	5.98737	0.05	**N.S.**
	L2 L5	2.61658	5.98737	0.05	**N.S.**		L2 L5	38.8829	35.5074	0.001	***
	L3 L4	0.29887	5.98737	0.05	**N.S.**		L3 L4	1.63504	5.98737	0.05	**N.S.**
	L3 L6	1.12625	5.98737	0.05	**N.S.**		L3 L6	91.5344	35.5074	0.001	***
	L4 L7	119.273	35.5074	0.001	***		L4 L7	34.4183	35.5074	0.01	**
	L5 L6	3.50761	5.98737	0.05	**N.S.**		L5 L6	4.25432	5.98737	0.05	**N.S.**
	L5 L7	0.01096	5.98737	0.05	**N.S.**		L5 L7	7.45249	13.7450	0.01	*
	L6 L7	6.19762	13.7450	0.01	*		L6 L7	42.4510	35.5074	0.001	***
Crude fat	L1 L2	150.968	35.5074	0.001	***	Crude protein	L1 L2	20.5931	13.7450	0.01	**
	L1 L3	435.961	35.5074	0.001	***		L1 L3	7.69765	5.98737	0.05	*
	L1 L4	1171.73	35.5074	0.001	***		L1 L4	15.3659	5.98737	0.05	*
	L1 L5	325.292	35.5074	0.001	***		L1 L5	1.37557	5.98737	0.05	N.S.
	L1 L6	539.081	35.5074	0.001	***		L1 L6	19.0389	13.7450	0.01	**
	L1 L7	165.125	35.5074	0.001	***		L1 L7	32.5681	13.7450	0.01	**
	L2 L3	2.20962	5.98737	0.05	N.S.		L2 L3	1.33630	5.98737	0.05	N.S.
	L2 L4	24.8206	13.7450	0.01	**		L2 L4	0.18790	5.98737	0.05	N.S.
	L2 L5	7.26146	13.7450	0.01	**		L2 L5	31.1239	13.7450	0.01	**
	L3 L4	38.8028	35.5074	0.001	***		L3 L4	1.91879	5.98737	0.05	N.S.
	L3 L6	0.41558	5.98737	0.05	N.S.		L3 L6	1.09311	5.98737	0.05	N.S.
	L4 L7	393.065	35.5074	0.001	***		L4 L7	3.88614	5.98737	0.05	N.S.
	L5 L6	75.2346	35.5074	0.001	***		L5 L6	154.043	35.5074	0.001	***
	L5 L7	9.50742	13.7450	0.01	*		L5 L7	165.758	35.5074	0.001	***
	L6 L7	89.0441	35.5074	0.001	***		L6 L7	17.5575	13.7450	0.01	**

Table 2b. Analytical statistical indicators

Persued parameter	Lots to compare	F crit.	F_α	Level	Statistical significance
Easily hydrolyzable nitrogen	L1 L2	0.42426	5.98737	0.05	**N.S.**
	L1 L3	6.16085	5.98737	0.05	*
	L1 L4	2.2142	5.98737	0.05	**N.S.**
	L1 L5	148.133	35.5074	0.001	***
	L1 L6	3.19333	5.98737	0.05	**N.S.**
	L1 L7	335.346	35.5074	0.001	***
	L2 L3	7.27089	5.98737	0.05	*
	L2 L4	3.5861	5.98737	0.05	**N.S.**
	L2 L5	243.252	35.5074	0.001	***
	L3 L4	2.18239	5.98737	0.05	**N.S.**
	L3 L6	1.06622	5.98737	0.05	**N.S.**
	L4 L7	54.9284	35.5074	0.001	***
	L5 L6	18.9243	13.7450	0.01	**
	L5 L7	1.91178	5.98737	0.05	**N.S.**
	L6 L7	27.8335	13.7450	0.01	**

CONCLUSIONS

Humidity increases in value, for pollen stored at 20°C and natural light.

The amount of organic matter showed the greatest decrease after 6 months if stored at 20°C pollen and natural light.

Increased amount of fat after 3 months and 6 months, showed changes in the pollinic membrane and the release of a rising amount of fat.

Dynamics of protein values was a downward after the first three months, followed by a slight increase after 6 months.

Easily hydrolyzable nitrogen (ammonia) showed the highest value for pollen stored at 4°C in the absence of light.

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