

PHYSICO-CHEMICAL COMPOSITION OF APILARNIL (BEE DRONE LARVAE)

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Abstract

This paper contains a summary about chemical composition of apilarnil (drone bee larvae), determining moisture, ash, free acidity, total protein and sugars content. Apilarnil is widely used in commercial medical products and cosmetics in many countries. The composition of apilarnil is quite complex and is not detailed in the literature. Seven samples of apilarnil were analyzed and compared with literature data regarding the physico-chemical parameters. This preliminary study presents a basic battery of tests for quality of apilarnil, which may be used as food supplement or in other domains.

Key words: apilarnil, carbohydrates, proteins, ash

INTRODUCTION

Apilarnil is a biologically active bee product. It is obtained by trituration and filtration of bee drone larvae, harvested on 7th day larvary stage, before the capping of the cells.

In Romania, Apilarnil was first made by Nicolae V. Ilieșiu, in 1980, when chemical synthesis of medicinal drugs was in the highest development. And yet, due to it's strong biological actions at that time, the product had a huge impact in scientific media. Name of APILARNIL was created by the romanian scientist, using more abbreviations: API from latin bees, LAR from larvae and his initials name NIL (Nicolae Iliesiu).

Many papers, referates, monographs which promote this product were written, the invention being protected by patenting, being recognised in many countries of the world and receiving many medals on national and international level. But in time, this product was unfairly forgotten or put in obscurity, when its place would be among the top products of the hive.

Apilarnil is a homogenous and milky substances, with yellowish grey colour and sour taste. It is easily adulterated and in its

raw form, which is why it has to be stored in the freezer.

Physico-chemical composition is similar to royal jelly, but yet different.

It was reported that Apilarnil contains 25-35% dry matter, 9-12% proteins, 6-10% carbohydrates, 5-8 % lipids, 2% ash and 3% unidentified substances [8; 10]. In addition, Apilarnil is rich in male type hormones so, it has many male strengthening effects [3]. It was suggested that apilarnil is a natural anabolism stimulator in males since, it increase the muscular body weight [10]. Also in the chemical composition of Apilarnil we found vitamins (A vitamin, betacaroten, B1, B6, PP and choline), minerals (calcium, phosphorous, sodium, zinc, manganese, iron, copper and potassium).

The main components of bee larvae are the aminoacids. Bee larvae contain all essential aminoacids, the only source being food products, as they can't be synthesized by human or animal organisms. For this reason we think that would be interesting to evaluate Apilarnil for chemical composition and biological activity, this product being so similar to royal jelly, the crown product of the bees.

The main objective of this paper is thus to summarize the chemical composition of fresh Apilarnil.

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MATERIAL AND METHOD

Fresh Apilarnil samples were used for the present study, harvested from Transylvanian beehives in conditions of hygiene and transported in cooled dark containers until the laboratory for analysis. The time from harvesting and final storage place at -18°C was every time up to one hour.

Water content: In a glass capsule with flat bottom of 5-6 cm diameter, 1g of each apilarnil sample were placed and dried in an oven at 60°C temperature for 3h until constant mass. Water content was determined gravimetrically by subtracting the final weight from initial weight and reporting to 100g of sample [1; 7].

Ash content: A known quantity of sample was placed in a clean crucible and placed in the incineration oven at 550°C for 6h, until a white powder was obtained. The crucible was weighted at the beginning and at the end and the difference was expressed as percentage of ash content.

Acidity determination: Acidity was determined from an aqueous solution 1% by automatic titration (TitroLine Easy) with 0.1N NaOH. Results were expressed as ml NaOH/g sample.

Total protein content: The total protein content was determined spectrophotometrically using the Lowry method [4], slightly modified and adapted for this bee product.

Sugars content: sugars content determination was performed by high performance liquid chromatography coupled with refractive index detector (HPLC-IR) using the method described by Sesta [9].

HPLC-IR analysis of sugars from apilarnil was made using a stainless steel column Alltima Amino 100Å (4.6 mm diameter, 250 mm length, 5 µm particle size). Temperature inside the column was kept at 30°C . Injection volume was 10 µl. Mobile phase was a mixture of acetonitrile: water (80:20. v/v) with 1 ml/min flow.

1g of apilarnil was mixed with 3 ml water: methanol (3:1. v/v) in a volumetric flask of 5 ml. After homogenization, 1 ml Carrez I reagent (distilled water solution of potassium hexacyanoferrate (II), $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$, 15 g/100 mL) was added, the mixture was shaken

vigorously, and 1 ml Carrez II (distilled water solution of zinc acetate, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, 30 g/100 mL) was added also and the volume was adjusted up to 5 ml. The solution is centrifuged for 30 minutes at 4000 rpm and the supernatant is collected and pre-washed with dichloromethane for 2-3 minutes. The upper layer is collected and the procedure is repeated 3 times. The reunited supernatants are then filtered (0.45 µm) and injected into the HPLC-IR system. Sugar standards (glucose, fructose, sucrose, maltose, trehalose, isomaltose; Sigma-Aldrich St. Louis, USA) were dissolved in ultra pure water (1 mg·ml⁻¹ solution), mixed in equal volumes and diluted to perform the calibration curve on HPLC. Each standard was injected separately, to register the retention time and than in mixture, to see if all standards were baseline separated. Quantification was obtained by peak integration in comparison with standards. Results were expressed as g % for each sugar.

Statistical analysis: Analysis was performed in triplicate. Data were expressed as mean ± standard deviation, using Origin Software.

RESULTS AND DISCUSSIONS

The results for pure apilarnil samples are presented in Table 1 for the proximate analysis and in Table 2 are shown the results regarding sugars content.

Water content for the studied samples ranged between 69.70% and 76.44%, and a medium value of 72.06%. Comparing this parameter with royal jelly's water content, one can notice that for apilarnil the value is higher than in royal jelly. Wytrychowski et al., (2013) [11] studying a lot of 500 samples of royal jelly, found a mean value of $65.3 \pm 1.52\%$ for water content.

Ash content was under 1%, except 3 samples for which the values were higher than 1% (1.01% for A1, 1.13% for A5 and 1.00% for A6). Stangaciu (1999) [10] obtained values regarding ash content similar with our findings (2%). Garcia-Amoedo and Almeida-Muradian (2007) [2] analyzing royal jelly samples, obtained values within the range 0.93%–1.17%, similar with our Apilarnil determinations.

The acidity measured in our samples varied between 1.58 and 3.28. In the literature, it is stated that the acidity of the same solution of Royal Jelly (same concentration), was between 3% and 6% [8]. Regarding Apilarnil, literature studies are poor or missing regarding this parameter.

Total lipid content, ranged in the analyzed samples between 1.29 and 4.51%, with a mean of 3.8%. Literature studies reveal that lipid content of royal jelly is situated between 3 – 8% [8].

Total protein content was situated between 4.55% and 9.95%. Literature studies reveal that the content of total proteins is situated between 9% and 18% in royal jelly [8]. Apilarnil seems to have a smaller quantity of proteins in comparison with Royal Jelly (Table 1).

Two major sugars (fructose, glucose) were identified in all analyzed samples (Table 2, Fig.1). As it can be seen from Tabel 1 glucose content is more or less 10 times higher that fructose in Apilarnil samples. Sucrose was quantified only in one sample (A1 0.14g/100g). Minority sugars (turanose, maltose, isomaltose) were identified in all samples, and trehalose was identified in six samples. These sugars were also identified and quantified in literature, but only in Royal Jelly [9], our data being in the same range of values. Figure 1 presents the chromatogram of sample A4 regarding the sugar profile. Apilarnil shows the presence of isomaltose, a sugar which was not found in Royal Jelly [9]. More detailed studies must be done on more samples to verify this statement.

Table 1 Physico-chemical analysis of seven samples of fresh apilarnil

Samples	Moisture (%)	Ash (%)	Acidity (ml 0.1N NaOH/g)	Lipids content (%)	Total Protein content (%)
A1	73.88±0.88	1.01±0.02	2.11±0.64	5.19±0.03	7.82±0.64
A2	76.44±0.61	0.86±0.02	3.08±0.14	5.67±0.18	9.95±0.91
A3	72.54±1.06	0.98±0.02	2.15±0.41	3.19±0.08	6.64±0.32
A4	69.94±0.37	0.82±0.02	1.58±0.34	3.44±0.44	8.93±0.58
A5	71.29±1.11	1.13±0.07	3.28±0.41	3.33±0.45	5.21±0.07
A6	69.70±0.20	1.00±0.01	2.40±0.18	1.29±0.04	7.49±0.15
A7	70.64±0.78	0.80±0.01	1.68±0.31	4.51±0.57	4.55±0.34
Mean	72.06	0.94	2.32	3.80	7.23
SD (%)	2.42	0.11	0.65	1.47	1.92

Values represent means of three independent determinations ± standard deviations.

Table 2 Sugars content of seven samples of fresh apilarnil using HPLC – IR

Samples	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)	Turanose (g/100g)	Maltose (g/100g)	Trehalose (g/100g)	Izomaltose (g/100g)
A1	0.43	3.44	0.14	0.03	0.53	-	0.04
A2	0.14	1.30	-	0.01	0.24	0.42	0.01
A3	0.03	3.00	-	0.04	0.40	0.47	0.04
A4	0.69	7.27	-	0.24	0.09	0.09	0.42
A5	0.06	3.17	-	0.01	0.24	0.40	0.07
A6	0.15	3.37	-	0.01	0.42	0.39	0.10
A7	2.68	3.72	-	0.01	0.36	0.86	0.06
Mean	0.60	3.61	0.14	0.05	0.33	0.44	0.11
SD (%)	0.95	1.80	-	0.08	0.15	0.25	0.14

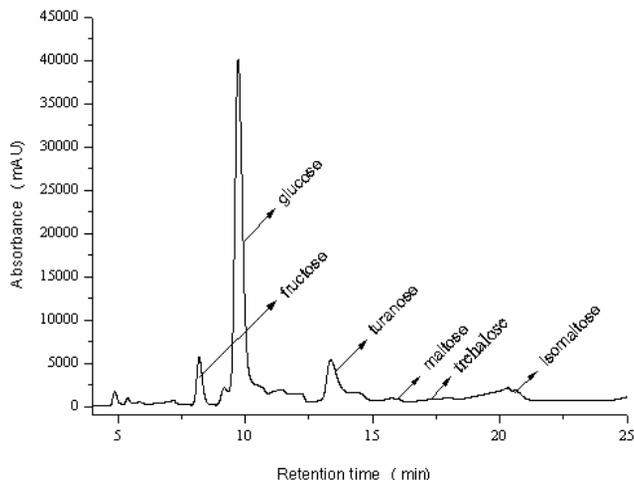


Fig 1. HPLC-IR chromatogram of apilarnil sample A4

CONCLUSIONS

The described methods are suitable and adequate for a routine quality control of Apilarnil, and represent a valid alternative to other more complex methods.

Sugars' profile determination, instead of global sugar analysis, is an important tool for bee practice and physicochemical analysis of Apilarnil.

Before going into more detailed parameters, all these analysis must be performed for every sample of apilarnil as a part of authenticity testing.

Transylvanian samples of fresh Apilarnil proved authentic and possess a high standard of quality. The most important statement in collecting and commercialization of Apilarnil is hygiene, storage and marketing conditions.

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