Summary

Studies and research on bee products with a view to their superior utilization

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The fundamental field of PhD: Engineering Science
The field of PhD: Industrial Engineering
The field of documentation: Beekeeping and the products obtained from this activity

STUDIES AND RESEARCH ON BEE PRODUCTS WITH A VIEW TO THEIR SUPERIOR UTILIZATION

Utilization in the shape of bee cocktails, functional food and cosmetic products on the basis of bee venom

SUMMARY

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THE STRUCTURE OF THE THESIS

The PhD thesis was worked out on the basis of research done throughout several years in my own apiary, then within the Apilife company and the collaborations with The Romanian Society of Beetherapy, with the Hofigal Society, with The National Institute of Research – Development for Cryogenics and Isotope Technologies in Râmnicu Vâlcea, with USAMV in Cluj, etc.

The paper is divided into seven chapters, conclusions, bibliography and annexes. It has 180 pages out of which the reference part represents 65 pages. The master's degree paper contains 32 figures and 16 tables, and the annexes are rendered in 28 pages. In order to

elaborate the thesis, there were used 100 bibliographical references. The dissemination of results is represented by the list of papers presented in national and international beetherapy Congresses and Conferences.

The PhD thesis is divided into two different parts:

I) The reference study
II) The experimental part

In the reference part, divided into two chapters (1 and 2), I deal with data in the specific literature: the definition, classification, physical-chemical composition, production, harvesting methods, packing and depositing of bee products analysed throughout this paper, namely: honey, raw pollen, maiden wax, propolis, royal jelly, drone larvae and bee venom.

In the experimental part, divided into four chapters (3, 4, 5 and 6) there is personal research concerning bee products with therapeutical use: personal research concerning bee products as raw material (chapter 3), bee cocktails (chapter 4), functional food with the products of the beehive (chapter 5) and cosmetic products with bee venom (chapter 6).

The paper ends with some conclusions and perspectives (chapter 7), bibliography, annexes, the list of notations and used symbols, the list of figures, the list of tables, my Curriculum vitae and the list of published or presented papers.

Before moving on to the presentation of the paper, I ought to thank and express my gratitude to those who supported me in elaborating and completing my master's degree thesis.

First of all, I want to thank God for everything I am.

I also want to thank PhD Prof. Eng.Vasile Jâşcanu, the scientific guide of the thesis, for his guidance and competence in coordinating my activity throughout the elaboration of the thesis. I also want to thank PhD Prof. Eng. Ioan Danciu for his time and support for completing and presenting the thesis.

Special thanks to Mrs. PhD. Pfarm. Gabriela Vlăsceanu for understanding, support and especially for the material and logistic support at my disposal, to Doctor Ştefan Stângaciu, to Mrs. Iuliana Crişan, …

I thank the board of examiners for assessing and presenting the master's degree thesis, for the honour of analyzing my thesis.

Last but not least, I want and need to thank my family: my mother for supporting me, my husband, our 11 – old month son, my dear friend Romeo for his support and understanding throughout the elaboration and completion of the master's degree thesis, for his moral support, for his understanding and unconditioned support throughout the research period.

I dedicate my success to them….

The aim and objectives of research

The aim of this project on "Studies and research on bee products. Their utilization as bee cocktails, functional food and cosmetic products on the basis of bee venom" is the documentation, experimentation and estimation of some technological alternatives of getting some complex food and cosmetic products based on products of beehive, in view of the optimization of the:

- chemical composition (by association)
- way of preserving
- effect on health

The research activity within the project aims to practically get three categories of bee – fit therapeutical products:

- bee cocktails
- functional food
- cosmetic products

and the results are stipulated to be of interest, both on the national and the international market, in the field of food supplies and cosmetic products based on products of the beehive.

Taking into account the importance of chemical compounds in bee products in keeping and improving health, as well as the curative characteristics of fruit and medicinal plants (analysed again and valorized with the help of modern technologies), we have in view to get some "bee-fit" therapeutically efficient products at the level of the human body.

Thus, the research aims the following:

I. The products of the beehive were studied in the first stage
   - Honey
   - Pollen
   - Bee bread
   - Propolis
   - Royal jelly
   - Drone larvae
   - Bee venom

considering the chemical composition, in certain compounds with benefits on the human body.

II. The second stage aimed to get some bee-phytotherapeutical products such as "bee cocktail"; and it had in view both the finding of some technological alternatives of production, and the discovery of the best ways of conservation and dosage.

III. The third stage was concerned with the study of the possibility of getting some functional food based on bee products, associated with fruit and medicinal plants, in order to keep and improve health.

IV. The fourth stage aimed to formulate and test a range of cosmetic products based on bee venom, simple or associated with medicinal plants.

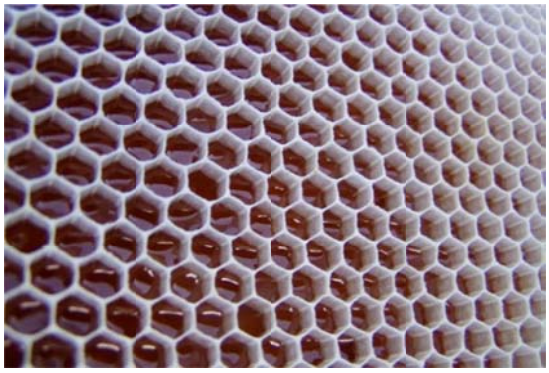
Chapter 1

1.1 Bee products, raw materials – general concepts

1.1.1 Honey

According to the dictionary, honey is a semiliquid substance, sweet and flavoured, rich in sugar and vitamins, made by bees from the nectar of flowers, but also from sweet secretions of trees after having been pricked by aphidae.

The nectar that bees suck from flowers is turned into honey through some sort of a "kiss" among bees (the kiss of bees) which participate in the process of maturation of honey through which small drops of nectar are transferred from one bee to another, this being the moment when it is enriched with the enzymes that ennoble it, then the water excess is eliminated. The production of honey by bees starts with harvesting the nectar or the manna and ends with putting lids on the cells of the honeycomb where honey was stored.



The processing is achieved through two ways: enzyme processing and hydric processing. The enzyme processing comes out of the necessity of bees to turn complex sugars (saccharose, maltose, etc.) into simple, assimilable sugars (glucose and fruitose) in order to completely assimilate them during wintering. The hydric processing is achieved in two stages: dilution of nectar or manna with saliva (the vector of sugary enzymes) then concentration. The purpose of concentration is to reduce the water content in order to obstruct the enzyme process, the activity of microorganisms (the osmotic pressure) and the decrease of the storage space.

At the moment of complete maturation of honey by bees in the beehive ("of three quartes lidded"), beekeepers take the frames out, put them in a clean room , take the lids off, centrifuge them and pack honey in big dishes.

Honey can be cleaned by possible foreign bodies: small wings, bees, larva, small wax lids, etc, by filtration or cleaning the above layer which appears after a short period of time.

The flavour of honey is given by the source-plant of nectar, from discret (acacia) to specific flavour of linden tree, raspberry bush, etc. up to bitter (the chestnut honey). **The taste** of honey is very sweet. **The colour** of honey may differ from almost colourless (acacia honey) to dark brown (manna honey). Good honey (16-18% humidity) is viscous at normal temperature (20°C). If **humidity** is higher than 21%, honey flows like water which means that it was inadequately obtained or kept, and it will degrade rapidly, being of low quality. **The density** of honey is strongly influenced by its content of water. Honey with 20% humidity has got the density of 1,4710. The acidity of honey is maximum 4 for flowers honey and maximum 5 for the manna one. The normal values of pH of honey are between 3,5-4,5, so the chemical reaction is a strongly acid one. **The electric conductivity** is one of the parameters which help to certify the authenticity of manna honey. The electric conductivity differs a lot from one range to another with floral honey. **The pollinic spectrum** is the main criteria for recognizing the origin of honey and the correct appreciation of the type of honey, due to the fact that the morphology of the pollen granules is absolutely characteristic for each species of plants. The pollinic spectrum of honey is the loyal mirror of its origin. Inadequately kept honey without a well-closed lid absorbs the smell around, and in a humid are it absorbs humidity. Honey consists of: water, carbohydrates, pollen, minerals, enzymes, vitamins, pigments, acid and aromatic compounds. **Carbohydrates** (sugars or glucides): fruitose (38%), glucose (31%), saccharose (1%), maltose and other disaccharides (7%), melezitose (sweet substance) (specific to manna honey). **Water** is between 16 – 23% and it is "biological", it comes from plants. **The nitrous substances** are expressed in protein equivalent and are found in a very small quantity, below 1%. The existing **vitamins**: thiamine (vitamin B1), riboflavine (vitamin B2), Nicol acid (vitamin B3), vitamin K, folic acid (vitamin M), biotin (vitamin H), pyrodixin (vitamin B6). **The aromatic compounds** terpenes, aldehydes, esters. **Acids**: gluconic, citric, malic, chihlimbar, formic, acetic, butyric, lactic amino-acids. **The pigments**: carotene, chlorophyll and the derivatives of chlorophyll, xanthophyll. **The pollen** (5%) is the one to whom the presence of some small quantities of vitamins, amino-acids, proteins in honey is due. **The enzymes** in honey are: the invertase (it converts saccharose into fruitose and glucose), the diastasis (it converts starch into dextrines), the glucose-oxidase, the catalase, the phosphatase. They have a double origin in the flower honey: vegetal (the existing enzymes in nectar) and animal (the enzymes in the saliva of small bees). The enzymes in the manna honey have a much higher origin (the enzymes in the sap of plants that feed the insects which produce the manna, those secreted by insects during the process of digestion, those produced by the mushrooms and alga which contaminate the drops of manna on plants and those added through the saliva of bees). The characteristics of honey, its chemical composition and quality is mostly given by the content of enzymes. Enzymes are labile substances, especially thermolabile. High temperature causes the irreversible nonintensification of enzymes. Among the existing enzymes in honey I mention: the invertase, the amylase, the catalase. The amylase (the diastasis) is the enzyme which catalyses the reactions of transforming starch into dextrines and further and it is an enzymatic indicator

of honey being the most resisting enzyme. The catalase is an enzyme which catalyses the reactions of decomposition of peroxides with oxygen release. The research of the catalase in honey helps to confirm the type of manna honey, the confirmation of thermic degradation, the making evident of installing the fermentating process in a chemical way. **The minerals** existing in honey are: potassium, sodium, calcium, magnesium, chlorine, sulphates, phosphates, silicium. The mineral substances in honey have a low content in floral honey, but high in the manna honey. There may be situations where the maximum limits are exceeded and this is due to the inadequate extraction and storage of honey (in dust), in the case of prolonged contact of honey with nonfood metallic surfaces (wraps). These substances are responsible for the extraordinary qualities of honey, so highly appreciated by all of us. The composition and therapeutical effects of honey are established by the plants visited by bees, namely their active principles.

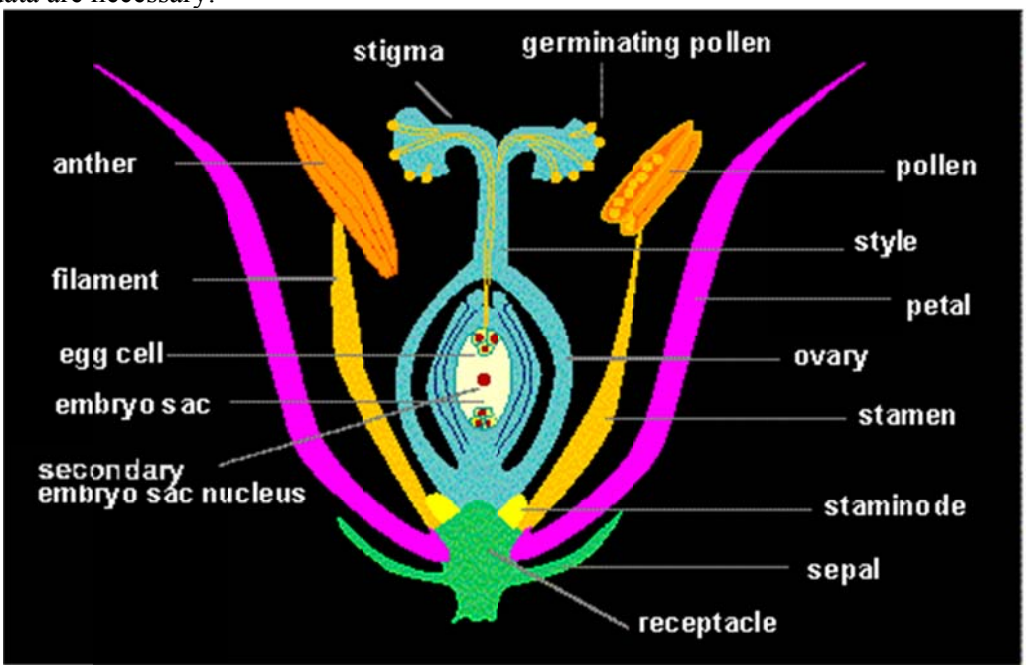


1.1.2 POLLEN

According to the dictionary, the pollen is a powder originating in the stamen of plants with flowers, which represent the male sexual cells.

For bees, pollen is their main source of food, the unique source of proteins, the main source of substances indispensable to their life: lipides, mineral elements, vitamins, enzymes, etc. For human beings, pollen is a source of vitamins, lipides, proteins, enzymes, minerals.

In order to understand the origin of pollen and the way bees pick up pollen, an image and some data are necessary.



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The role of a flower is to produce seeds. The essential female parts of a typical flower are: the ovary, the style and the stigma. The male part is the stamen. This produces pollen, which is the male sexual cell. The ovary becomes a fruit. The style is a prolongation of the ovary in the shape of a column. At the top of the style there is the stigma. With most plants the stigma has a sticky surface to which the pollen grains adhere where they germinate.

In order to give birth to the seed, the pollen has to reach the stigma from the anthers. Through germination, the pollen gives birth to a tube which passes through the interior of the stigma and the style reaching the ovary achieving fecundation. In the ovary fecundation takes place when the nuclei in the male germinal cell at the top of the pollinic tube join the nuclei in the female germinal cell.

Some plants produce a light pollen, this is transported by wind (anemophilous pollen), other plants produce a heavier, stickier pollen, and this is transported by insects (entomophilous pollen). 80% of pollination is achieved by melliferous bees. It confines itself to one species of plants in its flight, avoiding interspecific visits (Ialomiţeanu 1987).

Pollen harvesting by bees

There are some bots on the body of bees. The pollen attaches to the bots when the bee visits the flowers. The bee moves fast among stamens and the pollen attaches to the bots.



In the process of harvesting the pollen grains, bees go through several stages:

a. The pollen cells on the head, appendix and the first segments of the chest are “combed” by the hard bots of the front feet. The bots are moistened with nectar or with regurgitated honey to help packing the pollen. The hinder part and the last segments of the chest are cleaned by the bots on the medium pair of feet. The abdomen is cleaned with the bots on the hinder feet.

b. During the flight, the bee packs the pollen and stuffs it in the small baskets on the hinder

c. After having got in the beehive, the bee downloads the small baskets with the help of medium feet, in an empty cell or partly filled.

d. Behind it, there comes another bee which crushes and presses the pollen on the bottom of the cell with its head.



The pollen thus deposited is covered with a layer of honey, being to go through continuous changes biochemically turning into bee bread.

Pollen harvesting by the beekeeper

The pollen harvesting by the beekeeper takes place at the entrance in the beehive, at the bee entrance, through a series of devices called "pollen collectors" whose principle is a grate through which bees can pass only after having downloaded the small bags with pollen.

Thus the pollen falls into a collecting box which the beekeeper empties twice or three times a day in the wrap, then puts it into the freezer or in the pollen drying devices.



The organoleptic characteristics of pollen

When pollen is harvested, it looks like small grains with an irregular outline. The **colour** of pollen varies from white to black, the prevalent one being yellow. The colour varies a lot depending on the flowers visited by bees, for example: light yellow with hazelnut trees, dark yellow with the snap dragon, yellow lemon with rapeseed, greenish with maple trees, blue with facelie, light grey with elm trees, and almost black with poppy. **The taste** of pollen is pleasantly mellow, with an intensely similar perfume to the flowers it comes from. **The humidity** of pollen at harvesting is very high, that is why it is a very good environment for the development of microorganisms, that's why it must be rapidly processed (frozen or dried).

The size and the weight of pollen grains may differ a lot depending on the species of the plant, the duration of harvest and the distance to the source flower. The size and the weight of pollen grains count a lot on a commercial point of view. Crushed pollen does not look good to the customer's eyes. The size of the grains does not count on a therapeutical point of view, especially because it is recommended to be consumed in a mixture.

The physical and chemical characteristics of pollen. At the moment of harvest, the level of humidity of pollen is of 15-20 %, proteins 7-35 %, invert sugar 1-48 %, fat 1- 18%, mineral substances 1-7 %.

1.1.3 Bee bread



Bee bread, or “the bread of bees” may be the most valuable product of the beehive. Bees bring pollen in the beehive, deposit it in the cells, press it with their small head, mix it with little honey and enzymes and other nourishing substances from the salivary glands used as a binder for pollen grains , then they put a protective layer of honey. Here, under the influence of substances added by bees, of microorganisms, of high temperature and humidity in the beehive, pollen goes through a series of biochemical changes and structural modifications, turning into maiden wax after a period of natural effervescence.

Due to the fact that the changes of pollen are biochemical, the product obtained is different from the initial pollen. Turning of pollen into maiden wax involves several stages:

- the first stage consists of the development of a bacterium called *pseudomonas* , aerobian, which will consume the whole quantity of oxygen, and finally this leads to the death of these bacteria by selfsuffocation.
- the second stage is produced by the development of an anaerobic bacterium, *lactobacillus,* which uses glucides as the source of oxygen, producing lactic acid in exchange, its concentration reaches 3.2%; the concentration of vitamins B also increases.

Maiden wax is dark brown, it tastes sweet-bitter-sourish due to the process of effervescence.

The chemical composition: Maiden wax is rich in carbohydrates, proteins, vitamins, amino-acids and lactic acid for the food of bees. It is a natural product, more valuable than the pollen due to its higher content of simple sugars, vitamin K, enzymes and amino-acids, as well as to its increased acidity, which makes it easily assimilable. As compared to the pollen, its nourishing and antibiotic value is three times higher. The external cover of pollen, the exine, is also destroyed, leading to an easier assimilation by the body. The chemical composition of maiden wax is the following: carbohydrates (glucides 35%); lipides (1-6%); caretonoids (provitamin A 200-875 mg/kg; vitamin E 1.7 g/kg; vitamin C 6-200 mg/100 g of product.

Maiden wax contains more vitamin K and folic acid than the bee pollen.

1.1.4 Propolis



Propolis is a resinous material with the firmness of wax harvested by bees from *the buds* and *the bark* of trees. Its physical characteristics and colour differ and it is used inside the beehive for several purposes.

Propolis looks like a sticky mass of different colours, ranging from green, brown and black, having a flavoured smell of resin and balm. It is soluble in alcohol and ether and heavily soluble in water. Its specific weight is of 1.112 – 1.136 g/cm³. The melting point is between 70 – 120 °C. At 37 °C it softens, and at low temperatures it is breakable. If it is easily warmed in bain – marie, it splits up into two different parts:

- a viscous part which goes to the bottom
- a liquid part (propolis wax) which floats on the surface of water

The content of wax varies between 7,5 and 35 % (Ivanov, 1981)

The content of impurities varies between 18 and 34% (Makashvili, 1972)

The colour. It varies according to the geographical area and the plants of origin: light yellow, yellowish-greenish, yellowish-brown, brown reddish, dark brick-coloured, brown greenish, dark brown, etc. For instance, the prevalent colour of the Brazilian propolis is red, green or clear brown. **The smell**. The European propolis usually smells nice and mellow like the buds of poplars, wax, honey and vanilla. When it is burnt, it gives off a delicate smell due to the flavoured resin which becomes volatile. The smell of the Georgian propolis is generally similar to the propolis in other areas and regions of Russia (Makashvili, 1972). The Lithuanian propolis has a very powerful smell of cinnamon. **The taste**. Sour and sometimes bitter. The Georgian propolis has a bitter and prickling taste (Makashvili, 1972). Most of the Brazilian types of propolis are pungent. The flavonoides in propolis taste bitter and astringent (Jeanson and Marchenay, 1976). **pH** varies between 5.2 and 5.7 (Bracho et al., 1998). **The chemical composition**. Propolis is made of vegetal resin, balm of different compositions, wax, essential oil, iron, microelements, to which pollen, flavonoides, secretions of the salivary glands of bees are added. It represents a mixture of substances, especially: the flavonoide derivatives, the ferulic acid (active against Gram positive and Gram negative embryos), types of wax, amino-acids, balms, ferments, microelements (silicium, magnesium, copper, molybdenum, arsenic, tin, aluminium, vanadium, wolfram, iron, gold, iridium, calcium, cadmium, cobalt, strontium), antibiotic substances, resin, flavoured acids, acids. The composition of propolis varies according to the vegetal species from which it was harvested, but, on an average, it contains

- 55 % resin and balms,
- 30 % types of wax
- 10 % essential oil, quantities which are similar to any kind of propolis.

The production by bees. Bees collect a flavoured substance which looks like resin on at least 20 species of trees, especially on the poplar and alder-tree buds, on the leaves, buds and the bark of coniferous trees and poplars, Salicaceae and plum trees. The resinous materials thus collected are processed by specialized bees by mixing them with salivary secretions and wax.

The production of propolis takes place at the same time with the production of honey and is achieved bt specialized bees, on warm days, when the temperature is higher than 20°C, at a time when it turns into plastic. One can harvest a quantity of 100 – 400 grams of propolis from a beehive, according to the region.

1.1.5 Royal jelly

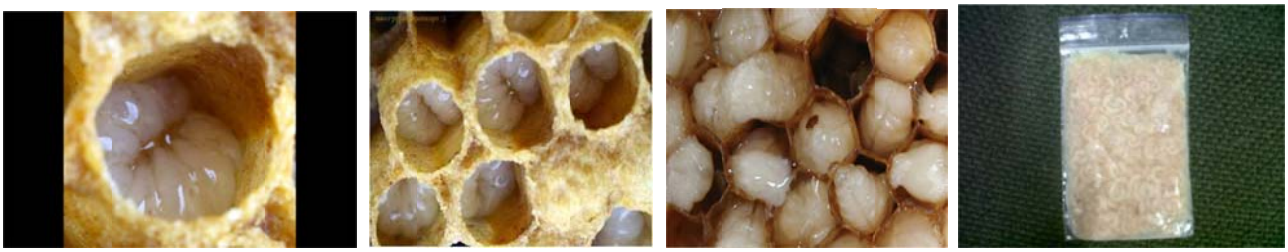



Royal jelly is a substance secreted by the hipofaringian glands of the youngest bees, on their 6th and 14th day of life, substance which will feed the queen and the sapling during the first three stages of larval development.

Royal jelly is an apparently white creamy and viscous substance. Its taste is specific, a little sour and astringent. It usually has a very stable composition, even the one obtained from different species or colonies of bees. Its stability may be the basis of the genetic stability of the colony of bees.The main components of royal jelly are: water, proteins, sugars, lipides and mineral salt (Rainer Krell, "*Value added products from beekeeping* , FAO, 1996). **Water.** It is about 2/3 of fresh royal jelly, but in the dried part, proteins and sugars are by far the highest fractions. A very important substance, existing in the royal jelly, is the 10 DHA acid. The concentration of the acid is a good indicator to check the quality of royal jelly. In the organic production of royal jelly, this must be higher than 1.8% of the dried material.

1.1.6 Drone larvae

Drone larvae is what is found and extracted of the cell where a future drone develops on the 7th day of larval stage, a mixture of larval common food (pollen, maiden wax, honey and water), representing 3%, the body of the larva drone, aged 7 days old and the larval covers which are then triturated, homogenized and filtered.
Drone larvae is an 100% Romanian bee product. Api= bee, lar=larva, n=Nicolae (Nicholas), il= Ilieşiu. Yes! The discoverer of this bee product is a great Romanian beekeeper, Mr. Nicolae Ilieşiu. It is obtained by extracting the whole content of the cell with the help of a pump.



There are three types of individuals in the beehive that form the colony: the queen, the working bees and the drones. The only female capable of laying eggs is, normally, the queen. It is the one that ensures the perpetuation of the species. It lays fecundated eggs out of which working bees or queens grow, and unfecundated eggs out of which drones grow.

The differentiation of the three castes is established by: the structure of the egg (fecundated or not), the qualitative and quantitative differenciated food and the environmental conditions (the plenty existence of the sources of nectar and pollen in nature, the strength of the bee family, the system of the beehive).

The eggs laid by the queen are fecundated or not according to the size of the cell where the queen lays the egg. If the cell in which the queen lays an egg is the normal one of honeycomb (4,6 – 5,1 mm), at the introduction of the abdomen it is pressed and it also opens the spermentom, a spermatozoon comes out which fecundates the egg, if the cell of honeycomb is bigger (6,4 – 6,6 mm), the spermentom does not open and an unfecundated egg falls on the bottom of the cell. An unfecundated egg – the diploid egg- contains 32 chromosomes (16 from the mature ovule, 16 from the spermatozoon), the unfecundated egg – the haploid egg- contains 16 chromosomes which hatch drones by parthenogenesis.

For a drone, the metamorphosis lasts 24 days: 3 days the stage of egg, 8 days the stage of larva, 13 days the stage of nympha.

In order to get the drone larvae, the beekeeper is especially interested in the period of the larval stage of the drone, namely the first 10 days from laying the egg, before the bees put lids on the cell. The best period of collecting drone larvae is on the 10th day after having laid the egg. On this day, the larva reaches the maximum development, the weight is a little below the maximum one, and the wet nurse bees laid a big quantity of food which helps the larva overtake the stage of nympha to the one of adult, in the lidded cell where only air and humidity pass. Starting from the weight of the egg 0,012 g it reaches the larval stage of 380 mg on the 11th day, and then an adult of 240 mg after 24 days. As compared to the weight of the egg laid on the first day, the larva becomes up to 1500 times heavier. This is caused by the feeding regime.

In literature two methods of collecting drone larvae are described. The first and the recommended one due to its superior quality is the one by extraction of the content of the cell with the help of a pump with vacuum. The second method is by squeezing the honeycomb, but the larval covers stay here.

The organoleptic characteristics of drone larvae. The aspect is homogeneous, a little viscous with fine granulations. The **colour** of drone larvae is white-grey ; the **firmness:** milky, unctuous,

slightly viscous. The **smell**: specific, slightly flavoured (a bit like the smell of fresh dregs); the **taste** : creamy, easily astringent; without impurities, parasites, fragments of larva.

The physical and chemical characteristics of drone larvae ph 4,8 – 6,7; the content of water 65 – 80 %; the content of dried substances 20 – 35%; total proteins 10 – 20 %; total glucides 1 – 5,5 %; lipides 5 – 6,3%; ash 1 – 1,5%; unidentified substances 4 -6 %; density 1,1 – 1,2%.

1.1.7 Bee venom

"The liquid bee venom is colourless, with pungent-bitter taste and flavoured smell similar to ripe bananas. It is slightly acid (pH 5.0 – 5.5); it changes the colour of blue litmus paper into red indicating an acid reaction. The specific weight is 1.313 g/cm³. However, the watery solution in the dried venom does not look like that, showing that the volatile compounds are responsible for the acid reaction. Venom gets dried at the environmental temperature in less than 20 minutes, losing between 65-70% of its original weight. When the liquid evaporates, 0,1 mg of pure dried venom can be collected (from only one bee)". (Michael Simics)

The bee venom is a combination of peptides, enzymes, lipides, amino-acids, carbohydrates with a powerful pharmacological action. The component which is found in the biggest quantity 40-50%, the one in which the quantity of venom is expressed is melitin. The hyaluronidaza is a valuable component enzyme which splits the hyaluronic acid. The apamin prevents the component C3 from developing and obstructs the channels dependent on potassium. Other substances, such as hyaluronidaza, phospholipase A2 and histamine are involved in the inflammatory answer of the venom. It also contains quantities of dopamin, serotonin and noradrenaline.

The bee venom is a product of secretion of the working bees, which is stored in the venom bag and eliminated outside at the moment of pricking, which is, in fact, a reflex action of defence. The secretion of venom is influenced by the age of bees, food and season. It has been established that recently hatched bees do not have any venom, only when they are 6 days old can they manage to get up to 0,15 mg, and when they are 15-20 days old, they get 0.30 mg.Bees brought up without pollen, so without proteins, do not produce venom. The generations of bees brought up in spring when the pollen resources are rich, produce more venom than those brought up in summer or autumn. Once used, the reserve of venom does not recover and the bee dies.


Chapter 2

Bee products and health

2.1 Therapeutical utilizations and actions

The origin, composition and characteristics of the main bee products lead to their curative features. Knowing them is extremely important in the therapeutical practice. The ratio substance – features is highly interesting for biochemists, chemists, biologists and researchers, the ratio features – pharmacological actions – therapeutic effect is very important for practitioners.

The bee honey is an excellent type of food with a high nourishing, biological and energetic value, easily assimilable, with real biostimulating features due to the content of natural antibiotic substances, ferments, vitamins and mineral elements.

The bee wax helps to make artificial honeycombs and as raw material in the electrotechnical, electronic, optic, cosmetic, pharmaceutical industries, varnishes and dyes, etc.

The pollen, propolis, royal jelly, drone larvae and bee venom are extremely appreciated for their therapeutical and biostimulating features.

The pharmacological characteristics of bee products are to be found in 10 pages of the thesis. 28 features of honey, 53 features of pollen, 60 features of propolis, 33 features of royal jelly, 17 features of drone larvae, 17 features of propolis were mentioned and justified.

2.2 The necessity of consuming food supplements (bee cocktail), functional food and the use of cosmetic products on the basis of bee products

The miraculous features of the mixture of bee products have been discussed lately, being possible to treat several diseases simply and rapidly. There is nothing better for the body than the organic, biological products which can be found around us in nature, and in order to prove this hypothesis, we can go back to our origins: the miraculous features of bee products were registered even in the antiquity.

Due to their components, bee cocktails are real “health sources”, taking into account the rich content in vitamins, minerals and other microelements, all these leading to its recommendation for multiple deseases. Being mixtures of beehive products in different formula, they help a lot our body which is so challenged by tiredness, stress, unhealthy food, pollution.

The concept connected to nourishment has expanded from survival and satisfaction of hunger to the prevention of contrary effects with the use of these beneficial products to improve the quality of life and to prevent or reduce the risk of being taken ill. In order to adopt a healthy lifestyle, we are in front of a new way of dealing with the nourishing science: the use of the so-called “functional food”.

Cosmetic products based on venom and other products of the beehive offer eternal youth due to the royal jelly (every day closer to a younger skin), the brilliance and delicacy of honey, the nourishing feature of pollen, the delicate texture of the natural bee wax, the cleanliness and protection given by propolis – the most powerful natural antimicrobial substance.

SECOND PART

Chapter 3

Personal research concerning the quality of bee products – raw material

3.1. Materials and methods

The analysis of the physical and chemical (a) and microbiological (b) composition of bee products which will be used in the bee therapy as functional food and in bee-cosmetics is extremely important because it is essential to know if products:

- are genuine,
- have the adequate chemical composition to the declared type of bee product
- do not have pathogenic microbial load, and the saprophytic load is in the limits of accessibility.

(a) For the bee products which will become part of the suggested api-phyto-therapeutical products, the main physical and chemical parameters were established: content of water, total proteins, total lipides (Table no. 6). The diastase index, electric conductivity and the content of HMF (hydroxi methyl furfural) were also established because other physical and chemical characteristics are also important for honey (Table no. 7). The working techniques used comply with the monographs in FR X and the European Pharmacopoeia, the valid edition.

(b) The study of the antimicrobial activity of some bee products consists in establishing the antimicrobial features of bee honey, propolis, royal jelly, as well as establishing the efficiency of these products according to the antibacterial qualities in view of using them in medical attendance. Thus, the objectives of the microbiological study aim two stages:

1) screening the antimicrobial activity of bee products as compared to different bacterial and fungi stems
2) the quantitative determination of the minimum inhibitory concentration of the bee products tested as compared to different microbial stems.

The biological material. The study was achieved on 16 isolated microbial stems from patients in hospital. The identification of the stems was achieved with the automized system VITEK 2, as well as with the help of the microtest galleries API 20. The standard bacterial stems used in the study are:

a) **Fungi stems**: Candida albicans 103101, Candida famata 366, Candida albicans 601,
b) **Gram-positive bacterial stems**: Enterococcus faecalis ATCC, Staphylococcus aureus MRSA 349, SCN, Staphylococcus epidermidis, MRSA 1840, Staphylococcus aureus ATCC,
c) **Gram-negative bacterial stems**: Escherichia coli 634, Escherichia coli 8730, Klebsiella 136202, Pseudomonas aeruginosa ATCC, Acinetobacterbaumanii 7706, Pseudomonas aeruginosa 6633, Pseudomonas aeruginosa 719.

The tested bee products were:

- Honey;
- Pollen;
- Royal jelly;
- Drone larvae;
- Propolis;
- Tincture of propolis;
- Soft extract of propolis

3.2 Results and discussions

(a) The main physical and chemical parameters of bee raw materials were established:

Table no. 6 The main physical and chemical parameters of bee raw materials used as raw material

No.	Product	Water content (%	Total proteins (%	Total lipides (%)
1.	***Acacia honey***	17.4	0.21	0.00
2.	***Manna honey***	16.4	0.68	0.01
3.	***Pollen 2013***	19.7	18.8	5.1
4.	***Pollen 2014***	19.2	18.6	5.8
5.	***Maiden wax***	15.7	16.6	4.4
6.	***Royal jelly***	63.7	13.4	2.2
7.	***Apilarnil***	61.4	7.6	3.2

The diastase index, electric conductivity and the content of HMF (hydroxi methyl furfural) were established for honey.

Table no. 7 Physical and chemical characteristics specific to honey

No.	Bee product	Electric conductivity (mS/cm)	Diastase index (DN)	HMF (mg/kg)
1.	***Acacia honey***	0.125	14.9	2.3
2.	***Manna honey***	0.962	36.9	4.9

As far as one can notice in the analysis, acacia honey and manna honey are genuine products, having a high diastase index (the content of amylase proceeded from bees) and very low content of HMF. The electric conductivity of manna honey is high, over the minimum limit of the Romanian standard and Codex Alimentarius so that a type of honey could be declared manna honey (0.8mS/cm).

For the main bee raw materials specific analysis were subcontracted by the laboratories of Physical-chemical and Microbiology Analysis of INCD IBA Bucharest (The Institute of Food Bioresources). The reports show the characteristics of raw materials (humidity, acidity, dried substance, total ash) according to valid STAS, but also pieces of information concerning the organoleptic features of bee raw materials: aspect, consistency, taste and smell, according to the monographs in the Romanian Pharmacopoeia, the valid edition (FR X).

b.1. Establishing the antimicrobial activity of different bee compounds upon the analysed bacterial stems.

A method adapted after the difuzimetrica one (Kirby-Bauer) was used.

Conclusions: 1. The antimicrobial activity of bee products appreciated through the difuzimetrica method was reduced, areas of inhibition of the growth being slightly highlighted, except the tincture of propolis.

2. It has been noticed that in some cases, the antimicrobial activity of the tested products increased by the continuation of the incubation period (from 24 hours to 72 hours).

b.2. Establishing the CMI value (the minimum inhibitory concentration) using the method of microdilutions in a liquid environment. It allows to establish CMI expressed in U.I/mgr or gram product.

Reading the results at spectrophotometer. The results were analysed by reading at spectrophotometer, too, this being a tool capable of measuring the quantity of photons accurately (the intensity of light) absorbed by putting them in a solution.
Thus, the quantity of substance (concentration) may be established indirectly.

For the stems of *Candida albicans* , all tested products have an antimicrobial activity, CMI has values between 1/10 and 1/80. The stem of *C. famata* proved to be sensitive only to the activity of pollen, the other compounds not having any effect. (**Table no. 7.1 and 7.2)**

Table no. 7.1 The CMI and CMEB values of the bee products tested on stems of *Candida sp*

	Drone larvae		**Royal jelly**		**Manna honey**	
Code of the stem	**CMI**	**CMEB**	**CMI**	**CMEB**	**CMI**	**CMEB**
Candida albicans 103101	1/80	1/10	1/320	1/320	1/40	1/40
Candida famata 366	1/40	No effect	No effect	1/10	No effect	1/80
Candida albicans 601	1/80	No effect	1/20	1/20	1/80	1/20

Table no. 7.2 The CMI and CMEB values of the bee products tested given the stems of *Candida sp.*

	Acacia honey		**Tincture of propolis**		**Raw pollen**	
Code of the stem	**CMI**	**CMEB**	**CMI**	**CMEB**	**CMI**	**CMEB**
Candida albicans 103101	1/40	1/10	1/320	1/10	1/80	1/40
Candida famata 366	No effect	1/20	No effect	1/10	1/160	1/10
Candida albicans 601	1/10	1/10	1/40	1/10	1/160	1/10

For the Gram positive stems analysed, drone larvae presented antimicrobial activity given all the stems, with an CMI value varying between 1/20 and 1/80µg/µl.

The rest of the other tested products had an activity only to certain stems, with various concentrations, between 1/10 and 1/320 µg/µl (raw pollen given the SCN stems) (**Table no. 8.1 and 8.2),** the narrowest spectrum of activity being in the case of royal jelly.

Table 8.1. The CMI and CMEB values of the tested products given the Gram positive bacterial stems

Code of stem	Drone larvae		Royal jelly		Manna honey	
	CMI	CMEB	CMI	CMEB	CMI	CMEB
Enterococcus faec ATCC	1/40	1/10	1/40	1/10	1/10	1/10
S. aureus ATCC	1/20	1/10	No effect	1/20	1/10	1/10
S. aureus MRSA 349	1/40	1/10	No effect	1/20	No effect	No effect
SCN	1/80	1/10	No effect	1/20	No effect	No effect
S. epidermidis	1/40	No effect	No effect	1/20	No effect	No effect
S. aureus MRSA 1840	1/40	No effect	1/20	1/20	No effect	No effect

Table 8.2 The CMI and CMEB values of the tested products given the Gram positive bacterial stems

Code of stem	Acacia honey		Tincture of propolis		Raw pollen	
	CMI	CMEB	CMI	CMEB	CMI	CMEB
Enterococcus faecalis ATC	No effe	No effect	1/20	1/10	No effect	1/10
S. aureus ATCC	1/40	1/10	1/80	1/20	1/160	1/10
S. aureus MRSA 349	No effe	1/10	1/40	1/10	No effect	No effect
SCN	1/10	1/10	No effect	No effect	1/320	No effect
S. epidermidis	No effe	No effect	1/160	No effect	1/320	No effect
S. aureus MRSA 1840	1/80	1/10	1/80	1/10	1/160	1/10

For the Gram-negative analysed stems one can notice that drone larvae and the tincture of propolis have an antibacterial activity as compared to all analysed species, and the activity of the other compounds varies according to the analysed stem.

The CMI values are between 1/10 and 1/320 μg/μl (**Table no. 9.1 and 9.2)**

Code of stem	Drone larvae		Royal jelly		Manna honey	
	CMI	CMEB	CMI	CMEB	CMI	CMEB
Pseudomonas aeruginosa ATC	1/80	1/10	1/40	1/10	1/10	1/10
Pseudomonas aeruginosa 719	1/80	1/10	No effect	No effect	1/10	No effect
Pseudomonas aeruginosa 6633	1/10	No effect	1/640	1/10	1/40	1/10
Acinetobacter baumanii 7706	1/60	No effect	No effect	1/10	1/80	1/40
E. coli 8730	1/40	No effect	No effect	1/10	No effect	No effect
E. coli 634	1/40	1/10	1/40	1/40	No effect	1/10
Klebsiella sp. 136202	1/80	No effect	1/10	No effect	1/10	No effect

Table no. 9.2 The CMI and CMEB values of the tested products given the Gram negative bacterial stems

Code of stem	Acacia honey		Tincture of propolis		Raw pollen	
	CMI	CMEB	CMI	CMEB	CMI	CMEB
Pseudomonas aeruginosa ATCC	1/20	1/20	1/10	No effect	1/320	1/10
Pseudomonas aeruginosa 71	No effect	No effect	1/10	No effect	No effect	No effect
Pseudomonas aeruginosa 66	1/40	1/20	1/10	No effect	1/160	1/10
Acinetobacter baumanii 770	No effect	No effect	1/10	No effect	1/20	No effect
E. coli 8730	No effect	No effect	1/10	No effect	1/320	No effect
E. coli 634	No effect	1/10	1/10	No effect	1/160	1/10
Klebsiella sp. 136202	1/10	No effect	1/160	1/10	No effect	No effect

Chapter 4

Bee cocktails (registered mark OSIM)

4.1 Processing raw materials and getting bee cocktails in the viewpoint of ISO 22000:2005

The analysis of the total polyphenols from the bee products used as raw materials in order to get **the standard formula of bee Cocktail** was achieved by using the Folin-Ciocâlteu method.

The content of total flavones was established by methods used in the specific literature , adapted to the analysis of bee products. Thus, **the Dowd method** was used, modified by Arvouet and col. (1994) and adapted by Meda and col. (2005).

Table no. 10 General bioactive principles of bee products and the bee cocktail product

No.	Bee product	Total polyphenols (mgGAE/10	Flavonoides (mgQE/100
1.	*Acacia honey*	17.71	4.4
2.	*Manna honey*	108.00	1.8
3.	*Pollen 2013*	563.2	255.1
4.	*Pollen 2014*	612.4	285.8
5.	*Maiden wax*	918.1	435.2
6.	*Royal jelly*	24.52	14.2
7.	*Drone larvae*	43.51	3.4
8.	***API PRODUCT***	**1056.3**	**532.6**

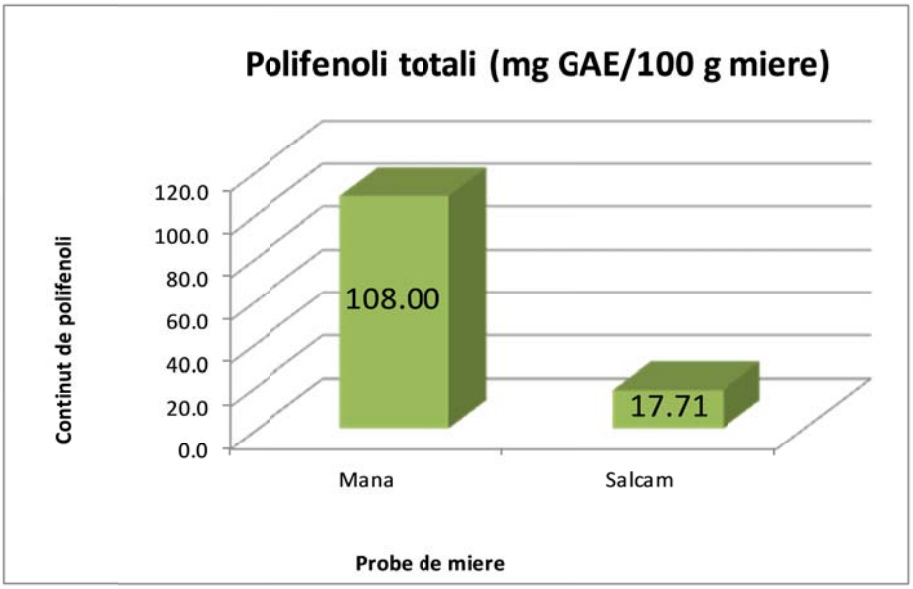


Figure no. 7 The content of total polyphenols of the types of honey analysed

As one can notice in Figure no.
1, there is a very big difference between the content of total polyphenols in the manna honey and the Acacia honey. The much more complex chemical composition, the dark colour of amber, the high content of minerals make the manna honey be an extremely valuable bee product, and establishing the content of polyphenols proves that.
The difference between the content of total flavones for the manna honey and Acacia honey is not so big, but the former contains about three times more flavones than the latter.

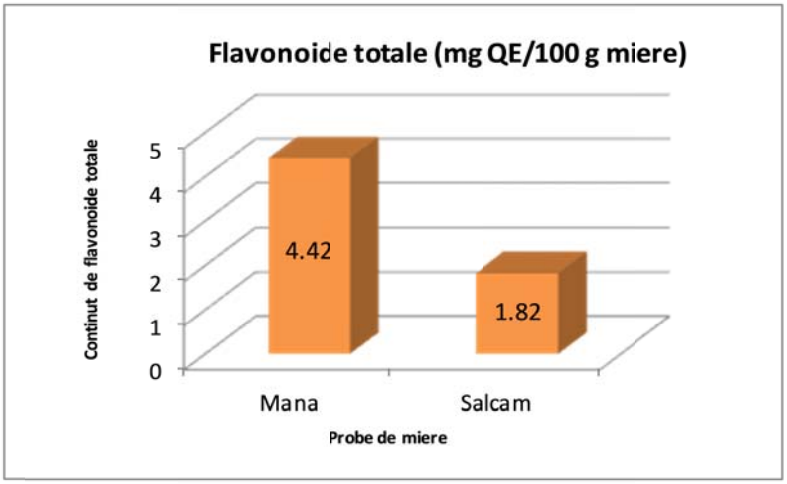


Figure no. 8 The content of total flavones of the types of honey analysed

The polyphenolic profile of propolis as well as the quantification of the identified compounds was achieved with the help of the high performance **c**hromatographical technique HPLC – PDA (**H**igh **P**erformance **L**iquid **C**hromatography – **P**hoto **D**iode **A**rray). Each signal in the chromatogram represents one or more polyphenolic compound, and these can be recognized by the time of retention, the UV – Vis spectrum and the maximum of adsorbtion.

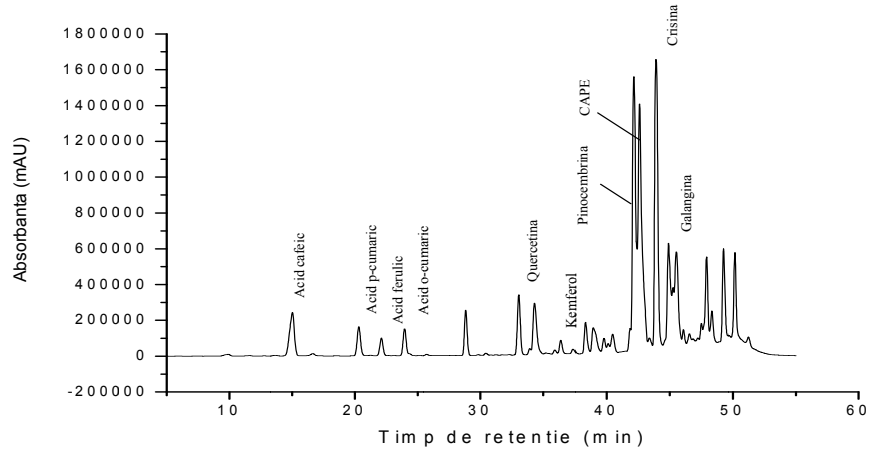


Figure no. 9 The HPLC – PDA chromatogram of the sample of alcoholic propolis

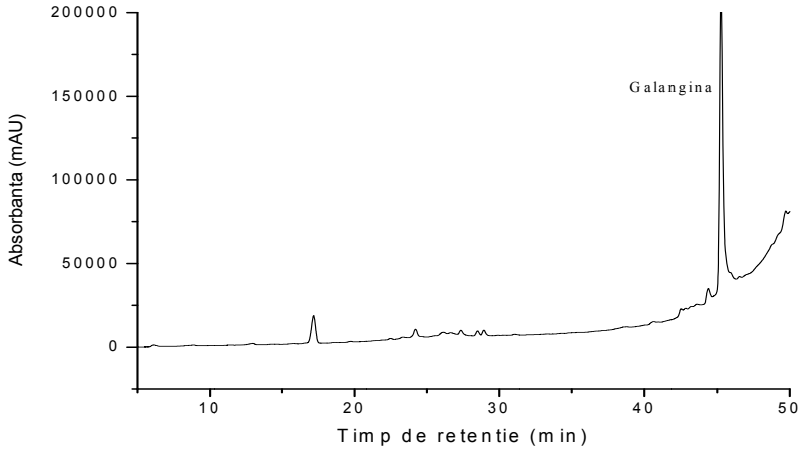


Figure no. 10 The HPLC – PDA chromatogram of the sample of watery propolis

Identifying and quantifying sugars is an important step with a view to establishing the authenticity of samples both from a quantitative and a qualitative point of view.

Table no. 11 The concentration of glucides (%) in the samples of bee products raw material and the apitherapeutical product achieved, established by the HPLC method

Sample	**Glucose**	**Fruitose**	**Saccharose**	**Maltose**	**Melezitoza (Sweet substance)**	**Total suga**
Acacia honey	23.77	41.00	1.03	1.44	0.00	**72.55**
Manna honey	26.25	35.14	0.14	1.77	1.78	**69.29**
Pollen 2013	13.02	18.29	0.00	0.1	0.00	**31.82**
Pollen 2014	15.20	16.27	0.00	0.10	0.00	**31.91**
Maiden wax	9.23	17.264	0.00	0.23	0.00	**28.66**
Royal jelly	6.67	6.16	0.51	0.15	0.00	**13.87**
Drone larvae	8.68	8.76	0.14	0.32	0.19	**19.00**
API PRODUCT	**19.49**	**27.08**	**0.01**	**0.95**	**0.47**	**50.21**

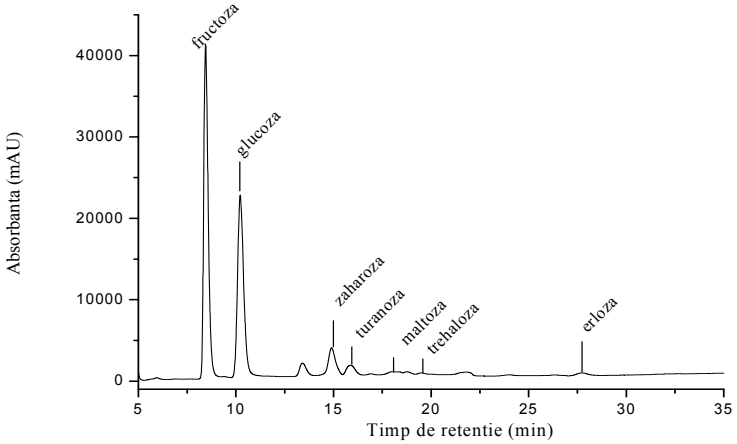


Figure no. 12 The HPLC – IR chromatogram of the sample of royal jelly

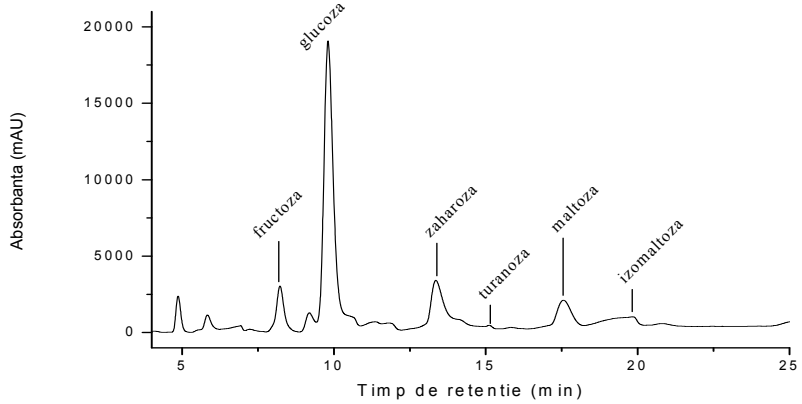


Figure no. 13 The HPLC – IR chromatogram of the sample of drone larvae

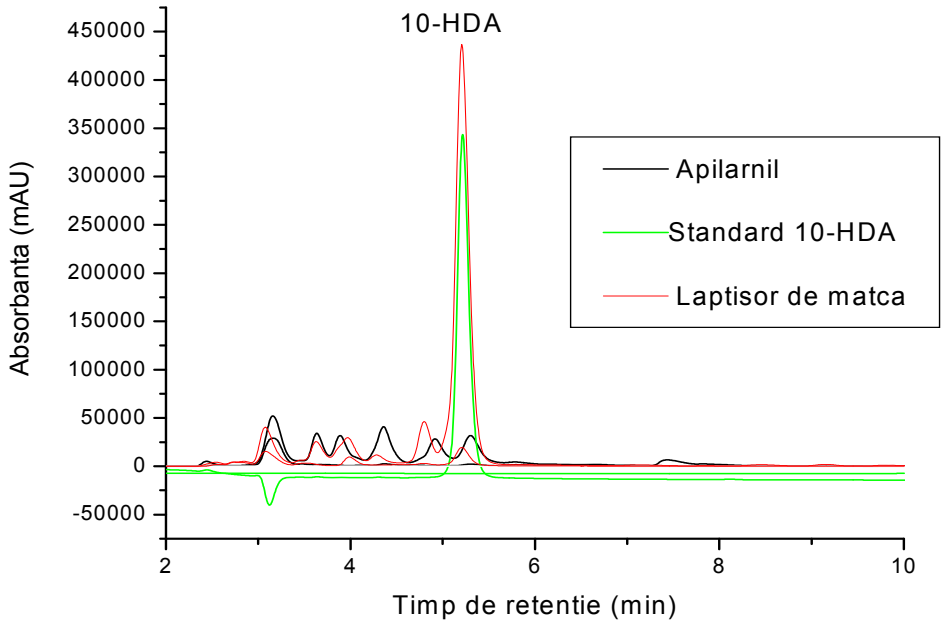


Figure no. 14 The overlapped chromatogram of the 10-HAD standard (40 mg/ml), of the royal jelly and drone larvae analysed

In the composition analysis of royal jelly, one intended to establish the content of 10 DHA acid (10-HAD), which is a marker of authenticity of the royal jelly.

Table no. 14 The antioxidant activity of bee products established by the DPPH and FRAP technique

No.	Bee product	DPPH (mmol TROLOX/g)	FRAP (mmol TROLOX/g)
1.	*Acacia honey*	0.10	0.25
2.	*Manna honey*	0.32	0.96
3.	*Pollen 2013*	1.37	2.47
4.	*Pollen 2014*	1.59	3.17
5.	*Maiden wax*	8.64	11.42
6.	*Royal jelly*	0.32	2.27
7.	*Drone larvae*	0.68	16.41
8.	***API PRODUCT***	**6.34**	**10.03**

As one can notice in the table above, both the total antioxidant potential and the activity of capturing the free radicals (DPPH) at bee products are high enough (as compared to other natural matrixes), and their combination in different ways leads to extremely valuable products such as their utilization in treating or even preventing various diseases.

The explanation of the beneficial activities of bee products and the recommendation of their use in apitherapy, or medicine in general, is given by the detailed clearing up of the chemical composition of each of them.

The variety of the composition is the decisive factor in the beneficial actions that these products have, the chemical analysis of their composition being their certificate of: guarantee, authenticity, quality. In order to comply with the valid GPM rules and the requirements for the safety of food, the ensurance of the traceability of the product from the beehive to the storehouse

or raw material, subsequently to its conditioning as a finite product as such or in different complex formula is required.

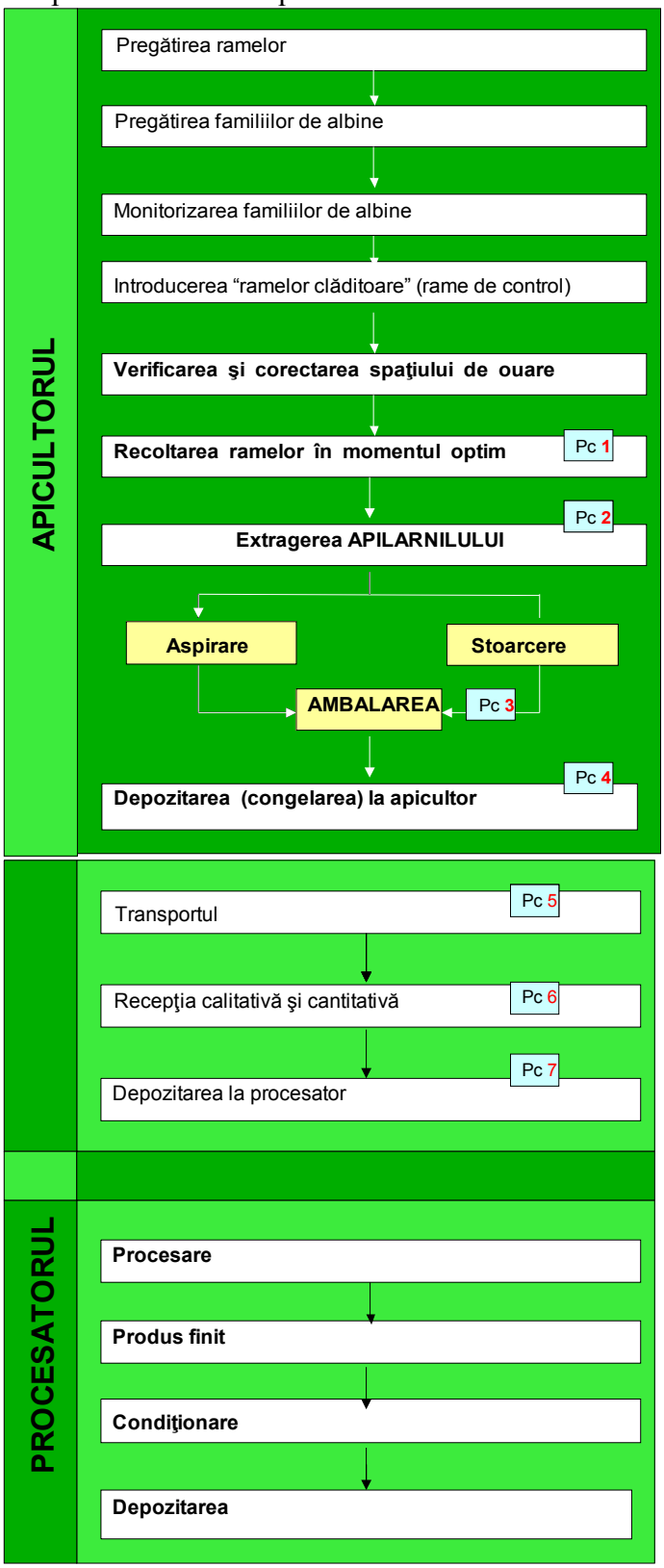


Fig no. 11 The traceability of Drone larvae from the beehive to the storehouse of raw material

Materials and methods

The quality of the drone larvae was checked by establishing: [1] the acidity – pH (5,9 – 6,9), [2] the humidity – water (65-75%), [3] the relative density (1,1 – 1,2), [4] the dried 10 g%), substance – residue (25 – 35%), [5] the ash (maximum 2%), [6] the reducing sugars (maximum [7] the total proteins (9-12 g %), [8] the total lipides (5-8 g %).

Results and discussions

In the process of getting the drone larvae, the authors appreciated that there are 7 checking points in all, out of which 4 checking points at the level of the operations performed by the beekeeper and other 3 checking points up to the moment where the drone larvae, as raw material, goes into processing.

Table no.13 Results of the physical-chemical analysis of Drone larvae (samples M vs. stored product P)

M	Proba	pH	Apa %	Densitate relativă	Rezidiu %	Cenuşa %
M	Apilarnil *1*	6.40	72.2	1.056	27.60	0.83
P	*Bihor*	6.30	72.0	1.060	28.00	0.85
M	Apilarnil *2*	5.98	68.4	1.080	36.60	0.83
P	*Bihor*	6.00	68.0	1.078	36.00	0.86
M	Apilarnil *3*	6.40	78.1	1.055	21.96	0.82
P	*Bihor*	6.30	78.5	1.060	21.50	0.80
M	Apilarnil *4*	5.30	73.00	1.025	27.00	0.50
P	*Giurgiu*	5.10	72.50	1.031	27.50	0.52
M	Apilarnil *5*	6.30	70.9	1.084	29.10	0.80
P	*Vaslui*	6.28	65.8	1.081	34.20	0.90
M	Apilarnil *6*	6.20	59.1	1.140	40.90	0.71
	Neamţ	6.10	57.2	1.170	42.80	0.72

Thus, from the first analysis, the supplier of the product Drone larvae 4 (Giurgiu) was eliminated because of the too high acidity of the product (pH 5.3), and the product Drone larvae 3 (Bihor) because of the too high humidity (78.1 %, compared to the limit of maximum 75% water indicated by the specific literature).Due to the high content of water, one could not establish quantitatively the total lipides of the samples: Drone larvae 2 (Bihor) and Drone larvae 6 (Neamț). The suppliers of the two products were eliminated because of the high quantity of residue established (36.6 % with Drone larvae 2 and 40.9% with Drone larvae 6, compared to the limit of maximum 35% indicated by the specific literature). Finally, the products Drone larvae 1 (Bihor) and Drone larvae 5 (Vaslui) were selected, the results of the physical-chemical analysis of these products being at the limits allowed.The product Drone larvae 5 became conspicuous due to a relative density a bit higher (1.084 compared to 1.056) and to a higher content of reducing sugars and total lipides (2.5%, respectively 4.9% compared to 1.7%, respectively 1.96%), and the product Drone larvae 1, from the point of view of the content of total protein.

The antioxidant activity of the bee products

Table no. 14 The antioxidant activity of the bee products established by the DPPH and FRAP technique

No.	Bee product	DPPH (mmol TROLOX/g)	FRAP (mmol TROLOX/g)
1.	*Acacia honey*	0.10	0.25
2.	*Manna honey*	0.32	0.96
3.	*Pollen 2013*	1.37	2.47
4.	*Pollen 2014*	1.59	3.17
5.	*Maiden wax*	8.64	11.42
6.	*Royal jelly*	0.32	2.27
7.	*Drone larvae*	0.68	16.41
8.	***API PRODUCT***	**6.34**	**10.03**

As one can notice in the table above, both the total potential antioxidant (FRAP) and the activity of capturing the free radicals (DPPH) with bee products is high enough (compared to other natural matrixes), and their combination in different ways leads to extremely valuable products for their use in treating or even preventing different diseases.

For the main bee raw materials, specific analysis were subcontracted by the laboratories of Phisical, Chemical and Microbiological Analysis of INCD IBA Bucharest (The Institute of Food Bioresources). The reports show the characteristics of the raw material (humidity, acidity, dried substance, total ash) according to the valid STAS, but also pieces of information concerning the organoleptic characteristics of bee raw materials: aspect, consistency, colour, taste and smell, according to the monographs in the Romanian Pharmacopoeia, the valid edition (FR X). See the **Annex no. 1** *Reports of the main bee raw materials*, **Annex no. 2** *HPLC Analysis for bee Cocktail*, **Annex no. 3** *Physical-chemical and microbiological analysis for bee Cocktail*

BEE COCKTAILS

The modern tendency is to use natural products both in prophylaxis and as treating adjuvants, in order to increase the therapeutical arsenal and to offer familiar, easily assimilable products to the body. Bee products are part of this category: honey, pollen, propolis, drone larvae and royal jelly which are components of the **BEE COCKTAIL.**

The mixture of bee products – BEE COCKTAIL is obtained by mixing different bee products: honey, pollen, propolis, royal jelly, drone larvae, maiden wax in different quantities selectively.

A system of dosage was established for the mixture of bee products, different systems of dosage were studied, with the remark that no other adverse reactions were noticed except the possible allergies.

4 bee cocktails are listed in the Api Life Ro portfolio, namely:

- Bee Cocktail of Immunity for Women
- Bee Cocktail of Immunity for Men
- Bee Cocktail of Immunity for Children
- Apilife Apicocktail

Chapter 5

Functional food with the products of the beehive

Generally, the products of the beehive represented for people, even in ancient times, not only a source of *food*, but also special *remedies* against different diseases. In antiquity, bee honey as the main bee product was recommended as "a primary food substance" and it was allowed to be eaten as having special characteristics, favourable to health and the prolongation of life.

Bee honey is a type of food which best combines the nourishing qualities with the therapeutical characteristics. The following question is asked: "Could bee honey be included in the category of functional food?" The answer would be: "Definitely, based on laboratory results and the integrative medicine, based on evidence." A supplementary reason: " In other countries, as well as in our country, honey and the other products of the beehive have entered the arsenal of traditional medicine for a long time".

ARONI ***APILIFE*** – a powerful antioxidant, it invigorates the immunity system, prevents the process of precocious ageing. It contains: bee honey with dehydrated fruit of Aronia melanocarpa (figure no. 19)

Bee honey combined with fruit of Aronia melanocarpa give the product ARONI APILIFE the characteristics of "food with specific use for keeping healthy" which:

- helps to invigorate the immunity system;
- has a beneficial effect on blood circulation, heart functioning and the normalization of blood pressure;
- reduces the oxidable stress and makes the process of precocious ageing slow down;
- it is recommended to pregnant women due to its rich content of folic acid;
- it is beneficial for the diabetes of type 2 due to the presence of inulin, it may decrease the level of glucose in the blood;
- it contributes to the detoxification of the body;
- it is an excellent general tonic

CĂTIN ***APILIFE*** – it fortifies the body. It contains: bee honey with dehydrated fruit of underbrush (figure no. 20)

Bee honey combined with the underbrush fruit gives the product CĂTIN APILIFE the characteristics of "food used to keep healthy" which:

- contributes to the completing of nutritive deficiencies;
- it is a general, vitaminizing, antianaemic tonic, a coronary, antiatherosclerosis protector;
- it helps children develop normally and harmoniously;
- it helps the body in stressful situations, nervous challenge, intellectual and physical effort, tiredness.

CAIS *APILIFE* – it ensures cardiovascular protection, fortifies the bony system, prevents ageing and the diminution of visual acuity, it is a good laxative. It contains: bee honey and dehydrated apricots. (figure no. 21)

Bee honey combined with apricots gives the product CAIS APILIFE the characteristics of "food used to keep healthy" which:

- normalizes the intestinal transit;
- supports cardiovascular health;
- prevents the appearance of cataract;
- prevents osteoporosis;
- has a tonic effect, removes physical and psychic asthenia.

The preparation of the product CAIS APILIFE as functional food was based on the remarks of some studies which proved that: "although dried, apricots keep their content of minerals and vitamins and, in winter, they get the value of medicine".

COACĂZ *APILIFE* – helps the body recover after intense physical and intellectual effort, stimulates the corticoadrenal function. It contains: bee honey with dehydrated fruit of black gooseberry bush (figure no. 22).

Bee honey combined with apricots gives the product COACĂZ APILIFE the characteristics of "food used to keep healthy" which:

- helps during the ……. cures by eliminating the toxins and the uric acid from the body;
- general tonic effect in conditions of asthenia, tiredness, for recovering after intense physical and intellectual efforts;
- stimulates haematopoiesis;
- it is a natural product with an effect similar to the cortizon-like hormones by stimulating the corticoadrenal function and releasing some substances with a remarkable anti-inflammatory and antiallergical effect;
- supports the normal functioning of the liver, the pancreas, the spleen and the kidneys; it improves the visual acuity.

GOJI *APILIFE* – gives energy and vitality, protects the body against oxidating stress, stimulates immunity, increases longevity. It contains: bee honey and dehydrated goji fruit (figure no. 23)

Bee honey combined with apricots gives the product GOJI APILIFE the characteristics of "food used to keep healthy" which:

- improves the disorders of eyesight, prevents the diminution of visual acuity, protects the retina.
- It is an important source of antioxidants which slow down the process of ageing;
- It increases the capacity of natural defense of the body;
- It regulates the level of cholesterol;
- It has a beneficial effect on conditions of anaemia due to its high content of iron, other minerals and vitamins;
- It protects the health of bones and ligaments, especially due to its high content of calcium;
- It is hepatoprotector and it has anti-ageing characteristics;
- It helps to balance the hormones, it improves the sexual function;
- It has a favourable influence upon conditions of anxiety, stress, tiredness and improves memory;
- Gives energy and vitality, it ensures good mood.

PRUN*APILIFE* – nourishing characteristics, an excellent digestive stimulus, an adjuster of the intestinal transit, a natural invigorator. It contains: bee honey and dehydrated plums (figure no. 24)

Bee honey combined with apricots gives the product PRUN APILIFE the characteristics of "food used to keep healthy" which:

- Is a powerful laxative and an excellent digestive stimulus which regulates the intestinal transit and normalizes the intestinal flora;
- Contributes to the regulation of the activity of pancreas and body weight;
- Encourages detox and cleaning the colon;
- Improves the liver congestion;
- It has nourishing characteristics, it is naturally fortifying and drives away the conditions of tiredness.

ZMEUR *APILIFE* – improves digestion, corrects the feminine endocrine disorders, keeps the silhouette. It contains: bee honey and dehydrated raspberry (figure no. 25)
Bee honey combined with apricots gives the product ZMEUR APILIFE the characteristics of "food used to keep healthy" which:

- Supports the normal functioning of the gastro-intestinal tract and improves the unpleasant symptomps of constipation;
- It is a way of maintaining the cardiovascular system, it prevents the arterial high blood pressure, the arterosclerosis.
- It reduces the appetite, being useful in preventing fatness;
- It has a positive influence upon the body sensitive to repeated colds, it decreases fever;
- It improves some feminine endocrine disorders, irregular period;
- It has nourishing, tonifying characteristics and vitamins.

MINT APILIFE – a powerful source of vitality, it stimulates digestion, controls diarrhea, conditions of nausea and vomiting. It contains: honey and fresh chopped leaves of mint (figure no. 26).
Honey combined with the chopped leaves of mint give the product MENT APILIFE the characteristics of "food used to keep healthy" which:

- controls abdominal distension, helps to eliminate the winds in the stomach and intestines;
- Prevents the fermentative intestinal process;
- It soothes conditions of vomiting and nausea;
- It controls diarrhea and normalizes the intestinal transit.

Results and discussions

Table no. 15 The content in active principles and microbial contamination for the samples of honey with powder of vegetal material (fruits and medicinal plants)

	Total no. o aerobian microorgan (TAMC) UI	**Total combine of fibreous lea and fungi(TY UFC/g**	**Enterobac ceae UFC/**	**Specific microorganisms**			**Content in pectines [%]**	**Content Expressed in chloride of , [mg/100g]**	**Content carotenes, expressed in** caroten, **[mg/**
				Staphylo coccus aur	**Escherichia**	**Salmonela spp.**			
The limit of admissibility	**10^4**	**10^2**	**10^2**	absent	absent	absent			
Honey with **Aro**	7,5 x 10^1	2,0 x 10^1 (Asp. flavus)	absent	absent	absent	absent	3,35	8,1	-
Honey with apri	5,0 x 10^1	0,5 x 10^1	absent	absent	absent	absent	-	-	-
Honey with **Underbrush**	< 10	< 10	absent	absent	absent	absent	3,32	-	3,94

Honey with Gooseberry	< 10	< 10	absent	absent	absent	absent	3,92	17,6	-
Honey with **Goji**	7,0 x 10^1	0,5 x 10^1 (Asp.niger)	absent	absent	absent	absent	2,91	-	0,65
Honey with plun	< 10	3,0 x 10^1	absent	absent	absent	absent	6,66	They identify; they can't be measured	0,008
Honey with Raspberry	1,1 x 10^1	0,5 x 10^1	absent	absent	absent	absent	6,91	2,2	-
Honey with Min	2,5 x 10^1	1,0 x 10^1	absent	absent	absent	absent	1,35	5,1	-

All samples are adequate. They joined the limit of admissibility stipulated by the valid European Pharmacopoeia. The samples of honey with Goji and Aronia join the limit of admissibility, but they are contaminated with Aspergillus niger. The samples are not contaminated with specific pathogenic microorganisms.

Chapter 6

Preparations with bee venom

Bee venom, raw material submitted to analysis

The characterization of the main proteinic fractions in the Romanian bee venom was achieved with the help of the high quality liquid chromatography and mass spectrometry. This approach was chosen in order to get valuable pieces of information (qualitative and quantitative) concerning the possible differences existing in the composition of the samples with bee venom with a different origin and collecting year.

The main aspects followed in the proteinic characterization of the venom were:

- stamp
- confirmation of the quality
- authenticity
- origin

Materials and methods

The origin of the venom samples was from Romania and Ukrainia, and they were collected in 2013. The samples were kept in the dark up to the moment of analysis, at a temperature of 5°C, and the data of identification of the samples were for:

V_1 –Gorj, Romania / **V_2** –Bihor, Romania / **V_3** –Sibiu, Romania / **V_4** –Neamţ, Romania / **V_5** – Ukrainia / **V_7** – Ukrainia.

The analysis of the venom samples with high performance chromatography of liquids, HPLC was done in the laboratories of The National Institute of Research-Development for Cryogenic and Isotropic Technologies – ICSI Rm. Vâlcea.

The quantification of the components of venom (melitin, apamin, phospholipase A2) was intended by using the method of the external standard using standards of reference of analytic purity, Sigma-Aldrich.

The samples of venom and the standards were prepared in acidulated water with TFA.

The chromatographic conditions were fulfilled by using:

- HPLC Thermo Finnigan Surveyor equiped with a detector row of diodes (PDA)
- Separation – the Aquasil C18 column (250 × 4.6 mm, 5 μm)
- PDA detector set at the wave length of 220 nm
- The elution in gradient of two mobile stages (water and acetonitryls acidulated with TFA – trifluoroactic acid)

Figure no. 27 High performance chromatograph of liquids, HPLC Surveyor Plus

The analysis of venom samples by mass spectrometry MALDI-TOF was intended to establish the profiles / proteic stamps by mass spectrometry. The working equipment was a mass Bruker Microflex LT spectrometer from Bruker Daltonics Inc. (*Billerica, MA, USA*) , with a support programme of control Compass 1.3 for FLEX v3.3 . The acquisition of mass spectra was followed by the statistical analysis of these. The purchased spectra were intercompared with those in the data base "Apitoxin Taxonomy" (*Ionete et al, 2013*).

Results and discussions

The HPLC method of establishing the proteic content of venom samples was applied to real samples, and the quantitative and percentage results are rendered in the Table no. 14.

Table no. 16 The quantitative and percentage composition of the active principles in the venom samples

Compus	Proba (mg/g)					
	V1	V2	V3	V4	V5	V7
Apamina	66.24	61.56	55.01	60.06	54.61	52.92
Fosfoloipaza A2	361.86	345.40	328.03	347.34	311.49	304.43
Melitina	830.78	762.55	746.45	809.07	790.81	760.07

Compus	Proba (%)					
	V1	V2	V3	V4	V5	V7
Apamina	4.5	4.7	4.1	4.5	4.0	4.1
Fosfolipaza A	21.7	23.4	21.8	23.1	20.1	20.6
Melitina	60.5	62.5	60.4	65.4	62.2	62.9

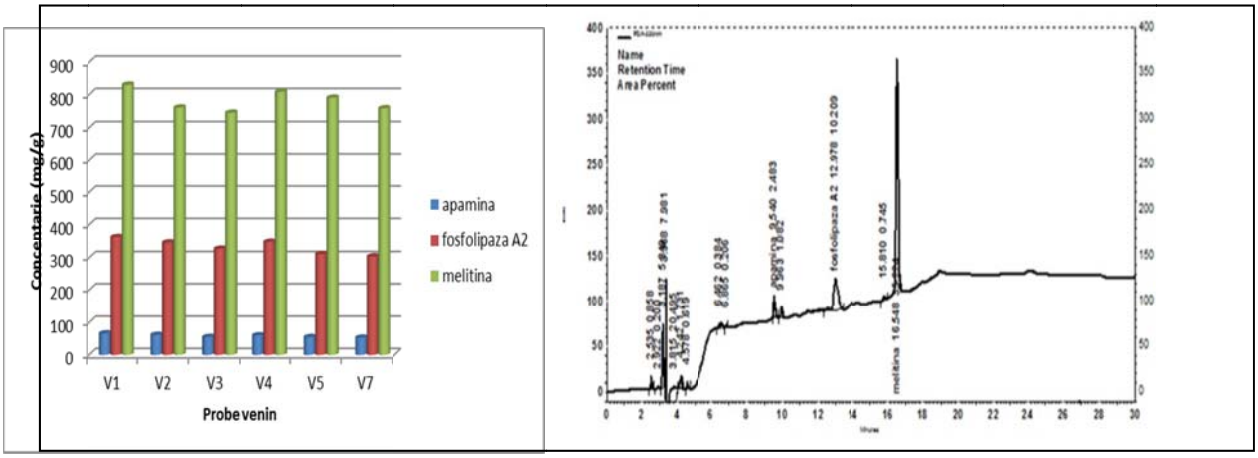


Figure no. 28 The composition of the venom samples and the HPLC chromatogram

The samples present a similar content of apamin, phospholipase A2 and melitin; an obvious separation of the components of apitoxin (melitin, apamin, phospholipase A2) is noticed compared to the integral venom (no matter if it is purified or in its natural condition, without purification – as it is collected by beekeepers) and to honey.

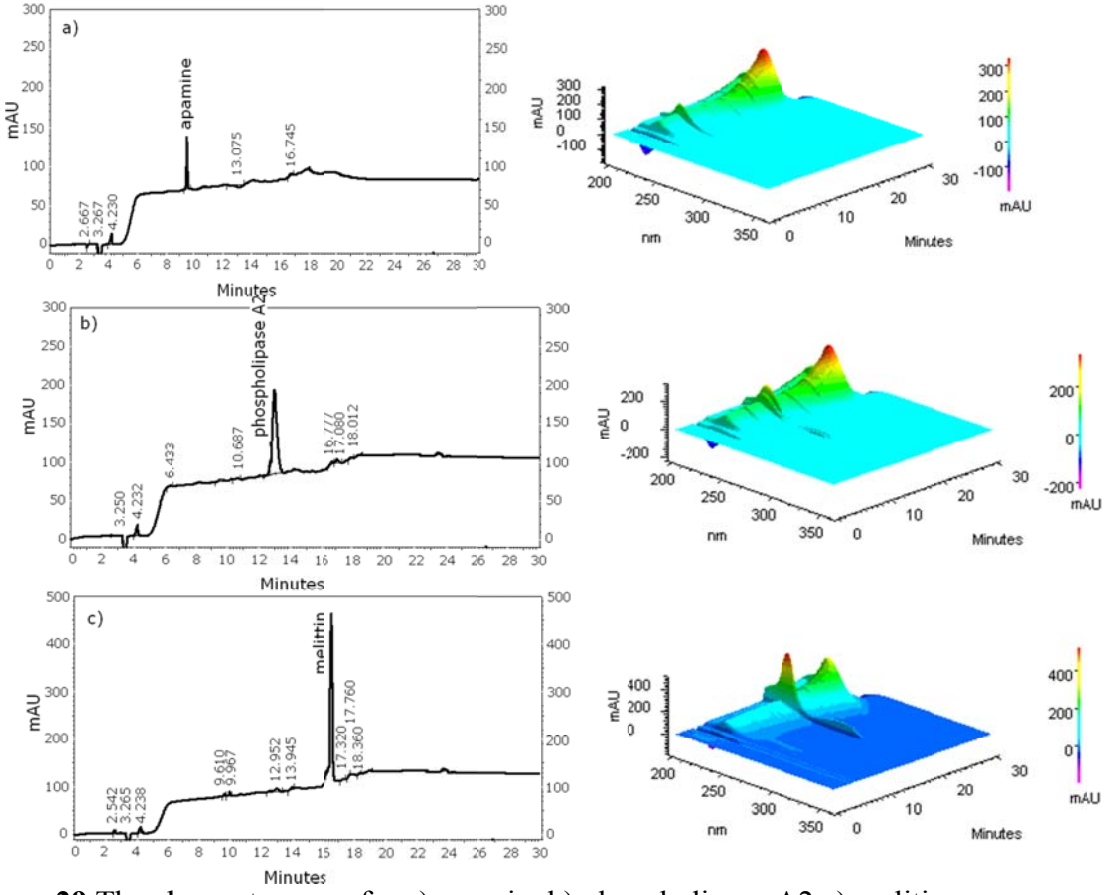


Figure no. 29 The chromatograms for a) apamin, b) phospholipase A2 c) melitin

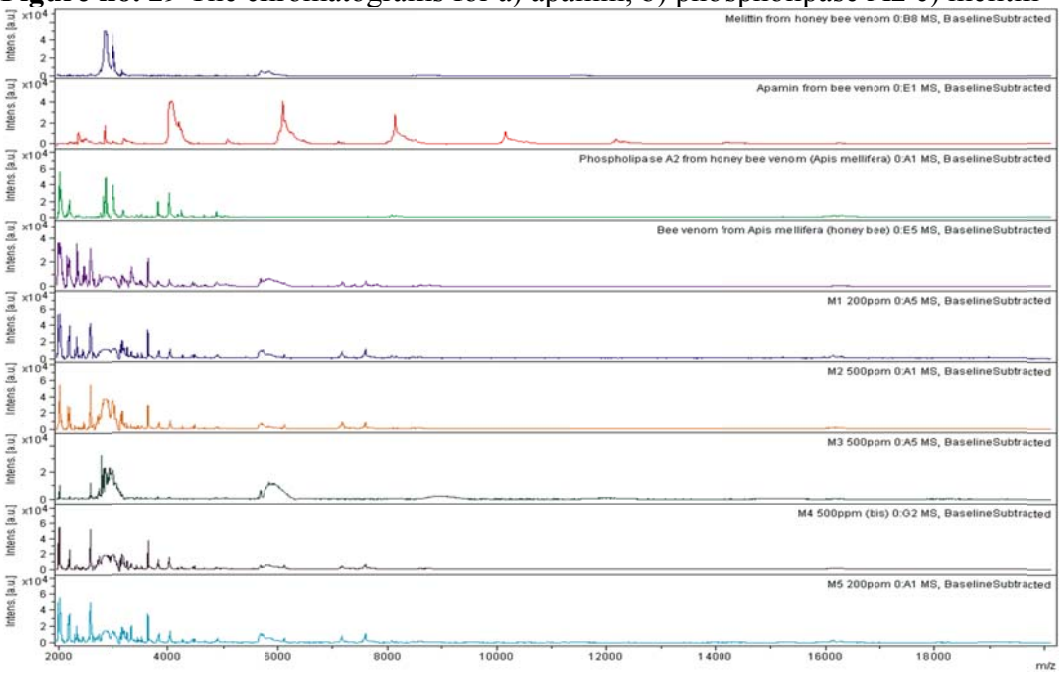


Figure no. 30 The selection of the most representative spectra in the multitude of measurements done for each analytic sample of standard and apitoxin

An obvious separation of the purified venom (*lyophilizated powder*) is also noticed compared to fresh samples of bee venom.

The analysis of venom samples by mass spectrometry MALD led to the obtained quantity of 0.6 mg/g for apamin, 11.4 mg/g for phospholipase and 9.3 mg/g for melitin.

Concerning the dispersion of the venom samples from beekeepers , one can notice their grouping in the same area of the diagram, without having a different separation among the venom samples – we can definitely say that it is natural (fresh) bee venom.

Practical results

We go on with the presentation of APILIFE cosmetic products

6.1 Revitalizing gel with bee venom, hot pepper, mint and pine

Ingredients (INCI): *Aqua, Glycerin, Capsicum frutescens extract, Carbomer, Triethanolamine, Mentha piperita oil, Pinus sylvestris oil, Benzyl alcohol and methylchloroisothiazolinone and methylisothiazolinone,* ***Bee venom***

Presentation: A special product, with bee venom, created to care and upkeep the skin liable to the process of biological and premature ageing of the body. The results of the local application led to the stimulation of the production of collagen and elastin, it helps to regenerate the skin, reducing fine lines and wrinkles, to increase the permeability of the capillary vessels and blood circulation, to decrease muscular, sciatic pains and to reduce neuromialgia , rheumatic and articular pains. The product is based on natural bioactive compounds: flavones, polycarboxyl acids, vitamins, phytohormones, enzymes, proteins, amino-acids, microelements, etc from the bee venom, the extracts of hot pepper, mint and pine essential oils with nourishing, antiinflammatory, soothing, revitalizing and emollient characteristics, in a base of gel which makes a very fine film/layer on the surface of the skin, thus preventing the loss of transepidermical water. Revitalizing effects, of rejuvenation of the skin, pain-killer, antiinflammatory and soothing effects for the cutaneous tissues and the inflamed joints, it gives suppleness, firmness and protection to the skin against the aggressive action of the harmful factors from the environment.

Action: Due to its rich content of natural chemical compounds from the bee venom, the extracts of hot pepper and mint and pine essential oil, the product helps the muscles and joints relax, it has an antiinflammatory effect, stimulates blood circulation, it is beneficial for the rheumatic muscular pains or due to other causes, neuralgia, bruises, strains, fractures, it keeps the resilience and the healthy aspect of the skin.

Way of wrapping up. The product is wrapped in jars made of plastic with a lid, of a capacity of 30 ml, 50 ml and labelled with the name of the product, ingredients, action, producer, graphic distinguishing marks, validity term, etc. The wraps have an elegant and modern design, ensuring at the same time the integrity of the product throughout the whole period of minimum durability.

Usage: Soft, local applications once up to three times a day on the affected area, in a thin layer. It absorbs rapidly and completely, it offers a feeling of freshness, contributing to the attenuation of the pain. **Notices**: Do not let it at hand and sight of young children. It is unadvisable to people with mycosis and inflammations. Bee venom must be avoided by asthmatic people, those allergic to bee stings, pollen and pregnant women. Before the first use, the product will be applied on a small surface of the skin (1-2 cm²), one has to wait for 48 hours and if there is no adverse effect, the product may be used according to the instructions.
Way of preserving: These products are to be used only externally and they are kept in a cold place, away from light and heat.

This product is the final, improved and notified in the European Union of the gel patented at OSIM with the application no. 2012 00285 on the 25th April 2012 the depositing date and published in BOPI 12/2013 , see Annex no. 7.

6.2. Regenerating nourishing face gel with bee venom and royal jelly

Ingredienţi (INCI): *Aqua, Glycerin, Carbomer, Royal jelly, Tiethanolamine, Parfum, Benzyl alcohol and methylchloroisothiazolinone and methylisothiazolinone,* ***Bee venom***
Presentation: A specially created product to care sensitive skin, liable to the process of wrinkles formation. That is why the skin must be cared correctly and efficiently. The product is based on bee substances, bee venom and royal jelly, rich in vitamins, phytohormones, a complex of enzymes, proteins, free amino-acids, microelements, etc. with nourishing, hydrating and emollient features, in a base of gel which makes a very fine film/layer on the surface of the skin, thus preventing the loss of transepidermical water, ensuring the transport of the nutritives in the deep layers of the epidermis, which gives suppleness, firmness and protection to the skin against the aggressive action of the harmful factors from the environment.
Action. It activates the cellular regeneration, feeds and fortifies the skin, activates blood circulation at the peripherical level, keeps the lipo-hydric balance and the pH of the skin in normal limits, prevents premature ageing and delays the natural ageing of the skin, reestablishes the firmness and the tonus of the skin. It has a fortifying, velvety, relaxing effect and increases the suppleness and firmness of the skin. It does not have unpleasant reactions upon the skin.
Way of wrapping up. The product is wrapped in jars made of plastic with an operculum lid, of a capacity of 15 ml, 30 ml, 50 ml, inscribed accordingly (the name of the product, the name of the producer, address, telephone number, fax, nominal content (ml), the sign of the recycling wrap, ingredients, an individual bar code, the number of the lot, the notice “To be used preferably before...”, conventional signs. The wraps have a special, modern design, ensuring at the same time the integrity of the product throughout the whole period of validity.
Usage: The product is for external use, it has to be applied in a thin layer, in the morning and in the evening, by a soft massage with the top fingers.
Way of preserving: The product is stored at temperatures between 10 and 25 °C, in its original wrap, in clean, dried spaces, away from heat, cold and humidity.

6.3 Solution of bee venom – spray


Ingredienţi (INCI): *Aqua****,*** *Capsicum frutescens extract****,*** *Mentha piperita oil****,*** *Pinus sylvestris oil****,*** *Benzyl alcohol and methylchloroisothiazolinone and methylisothiazolinone****, Bee venom***

Presentation: A special product, with bee venom, created to decrease muscular, sciatic pains and to reduce neuromialgia , rheumatic and articular pains. The product is based on natural bioactive compounds: flavones, polycarboxyl acids, vitamins, phytohormones, enzymes, proteins, amino-acids, microelements, etc from the bee venom, the extracts of hot pepper, mint and pine essential oil with nourishing, antiinflammatory, soothing, revitalizing and emollient features, in water, assuring the transport of phytocompounds in the deep layers of the epidermis.

Action. Due to its rich content of natural chemical compounds from the bee venom, extracts of hot pepper, mint and pine essential oils, the product helps the muscles and joints to relax, it has an antiinflammatory effect, stimulates blood circulation, it is beneficial for the rheumatic muscular pains or due to other causes, neuralgia, bruises, strains, fractures, it keeps the resilience and the healthy aspect of the skin.

Way of wrapping up: The product is wrapped in containers made of plastic with a capacity of 50 ml, with a sprayer and it is labelled with the name of the product, ingredients, action, producer, graphic distinguishing marks, validity term, etc. The wraps are easy to use and they assure the integrity of the product throughout the whole period of minimum durability.

Usage: Soft, local applications , once to three times a day, on the affected area, in a thin layer. It absorbs rapidly and completely, it offers a feeling of freshness, contributing to the attenuation of the pain.

Way of preserving: The product is stored at temperatures between 10 – 25 °C, in its original wrap, in clean, cool places, away from light and heat.

A survey on the irritability of the skin was done for all the three cosmetic products with bee venom, at ***The authorised Dermo Test Păuna SRL laboratory***, Annex no. 8.

Clinical surveys of testing the cutaneous tolerance from the point of view of the irritative and allergic reactions were done for all the three products with bee venom: ***"Revitalizing gel with bee venom, hot pepper, mint and pine", "Regenerating nourishing face gel with bee venom and royal jelly" and "Solution of bee venom – spray"*** on a lot of 20 people, healthy, nonallergic volunteers, men and women, the final report is found in the Annex no. 9.

Chapter 7

Conclusions and perspectives

7.1 General conclusions

The theoretical purpose of the thesis was to evaluate the qualitative (nourishing and therapeutical) value of the products of the beehive and, at the end, their practical utilization in the shape of preparations:

- Bee cocktails
- Functional food
- Cosmetic products

From the point of view of the food safety, it was proved that bee products, in order to be used in apitherapy, must strictly respect the collecting conditions, conditioning and storage. By their chemical nature (the main components being glucides, proteins, vitamins, enzymes) they are a favourable environment for the development of microorganisms, so they present the risk of microbial loading.

In order to respect the valid GMP (Good manufacturing Practice) rules and the requirements for the safety and security of food, one must ensure the traceability **of the bee product** from the beehive to the warehouse of raw material, subsequently to its conditioning as a finite product (as such or in different complex formula), by implementing an **Integrated System of Management** (of the food Quality, Environment, Safety and Security) on bee farms, in the factories of processing and producing bee products, which allows the notification and correction of risky factors by analysing the Critical points of control (Cpc).

The survey on ***"Studies and research concerning bee products. Utilization in the shape of bee cocktails, functinal food and cosmetic products based on bee venom"*** had in view the documentation, experimentation and evaluation of some technological alternatives to get some complex food and cosmetic products based on the products of the beehive, in view of the optimization of the:

- chemical composition (by association)
- way of preserving
- effect on health.

The importance of the chemical components in the bee products was taken into consideration to keep and improve health, as well as the curative features of fruits and medicinal plants (reinvestigated and used with the help of modern technology), having in view to get some "api-phyto" like products therapeutically efficient at the level of the human body.

We think that the aims of the survey were reached, having gone through all the stages established in the protocol of work (documentation, research, manufacture and test). Thus,

1) the most important products of the beehive from the point of view of the chemical qualitative composition were studied in the first stage, subsequently doing the quantitative analysis of the components with a beneficial effect on the body.
2) the aim of the second stage was to get some api-phytotherapeutical products such as "bee cocktail"; various technological ways of preparation were established and best ways of preserving and dosing were identified.
3) the third stage had in view to study the possibility of getting some functional food. Thus the range of functional food "APILIFE" was obtained, based on bee products which, in association with fruits and medicinal plants, help to keep and improve health.
4) the fourth stage, the last one, ended with the formulation and test of a range of cosmetic products based on bee venom, simple or associated with medicinal plants.

7.2 Recommendations

As a beekeeper, the author of this thesis has studied the methods of collecting bee venom for 10 years (2004 – 2014), noticing that the stimulation of the aggressiveness of bees establishes the growth of the collected quantity of venom. Based on her acquired experience, the PhD student Engineer Cornelia Abălaru (Doşteţean) is entitled to remark the following:

- the aggressiveness of bees can be stimulated without harming the physiology of the bee and the activity of the beehive
- the growth of the collected quantity of venom is directly proportional to:
- the power of the bee family
- the existence of sufficient resources of food in nature
- the use of the collecting technology of bee venom

recommending the improvement of the monitoring apparatus and the devices for collecting the bee venom in view of their optimization.

7.3 Personal contributions and future tendencies of research development

The PhD student:

- made the analysis laboratories of ICSI Romania, USAMV Cluj, ICD Beekeeping, INCD Food bioresources Bucharest, SC Hofigal Export Import SA Bucharest, etc. available, various samples of products of the beehive (from different places in the country and abroad, collected in different years) thus the main components being able to be analysed.
- being interested in the improvement of the technologies of getting bee products, especially in the collecting of bee venom and the permanent monitoring of its chemical parameters (melitin, apamin and phospholipase A2), she initiated the creation of a database and the implementation of a work method (validated by ICSI Romania) to analyse the authenticity and quality of venom (an essential stage in the standardization process of bee venom, necessary to all beekeepers and apitherapists because there is no Standard of product for the bee venom).
- by collecting the bee venom since 2004, she succeeded throughout the 10 years of experimentations and testing in improving the apparatus and method of harvesting, reaching the performance of 2014 of harvesting 2-3 g/day/20 families (compared to the first year when only 6 g/the whole bee season/ 20 families was harvested).
- as a legal representative of the economic agent SC Apilife Ro SRL Sibiu she got the registration at OSIM as trademark the product/the name “Bee Cocktail” in view of ensuring a better control of the market outlets of the mixtures of bee products with therapeutical use.
- by documentation and testing, she succeeded in perfecting the way of preparing bee mixtures like cocktail and functional food. By perfecting the way of conditioning raw materials (bee/fruits/plants) and the work method she succeeded in getting a stable, homogeneous finite product which no longer separates in phases.
- she created 3 cosmetic products based on bee venom which are now being notified by the European procedure harmonized on the portal CPNP under the commercial names of:
- *Revitalizing gel with bee venom, hot pepper, mint and pine*
- *Regenerating nourishing face gel with bee venom and royal jelly*
- *Solution of bee venom - spray*

and the dermatological testing done at an accredited laboratory classified the products in the category of Creams, emulsions, lotions, gels and oils for skin – face, hands, feet – with their classification in the category UNIRRITATING.

The purposeful practice of the research activity in the project was to get 3 *categories of* ***api-phytotherapeutical products*** :

- *bee cocktails*
- *functional food*

- *cosmetic products*

The results obtained are thought to be of interest, both on the national and the international market, in the field of

- *traditional food products*
- *food supplies*
- *cosmetic products*

based on products of the beehive.

We consider that due to the positive results obtained through this reference study of fundamental research with a practical purpose, from a scientific point of view, there are encouraging premises for other patent application to be registered at OSIM in the near future concerning:

- products
- procedures
- methods
- devices

in the field of beekeeping, food industry and api-phyto-therapy with a value at the national level.

For the reasons mentioned, after having presented her PhD thesis in a public meeting and its confirmation as being in accordance with the current legal conditions related to intellectual property, the PhD student will revise the study in the sense of mentioning in a wider and more detailed way several original aspects of the invention type.

The author also decided to continue and study thoroughly the research concerning the ways of getting bee products and their utilization in perfected complex mixtures as a nourishing and therapeutical contribution.

The results obtained will be communicated during national and international scientific manifestations, they will be disseminated with the help of printed publications and on computer in order to ensure a continuity of the research direction, by completing and bringing up-to-date with many other innovating, original and prior ideas, so that the general and endorsed public should have the possibility to apply directly and in a more pragmatic way all these scientific, theoretical and practical innovations which contribute to the progress of knowledge and social, bioeconomic and lasting development.

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