The Royal Jelly Book



For so work the honey-bees
Creatures that by a rule in nature teach
The Act of order to a peopled kingdom.
They have a king and officer of sorts.

Shakespeare, King Henry V

Shakespeare did not know, that the queen was the chief bee officer, royal jelly being the food stuff, produced by bees for raising her….

I would appreciate your feedback at http://www.bee-hexagon.net/contact/

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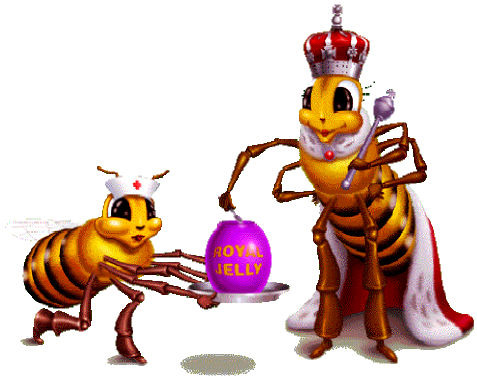
Royal Jelly and Bee Brood: Harvest, Composition, Quality

Stefan Bogdanov



THE SEARCH FOR QUEEN-MAKER IN ROYAL JELLY

Until the end of the 19th century royal jelly (RJ) was not known as a bee product. RJ is produced by the hypopharyngeal gland of young worker bees. In 1888 the German von Planta found, that the food of workers, drones and the queen was different, which was supported also by Haydak[22].

In the sixties and seventies an intensive research by Rembold and coworkers to identify of the key queen substance was carried out. It became clear, that the main components of the queen and the worker feedings , i.e. proteins, carbohydrates and lipids are the same, while royal jelly contains more amino acids, nucleotides and vitamines [61-63, 79]. The fatty acid pattern of the RJ is also different from the worker jelly [46], while the juvenile hormone, present in RJ plays also a role for the emergence of the queen [61].

Also, it was found that feeding of lower amounts of fructose and glucose produces workers, while feeding of higher amounts of these sugars produces queens [1].

Another hypothesis incorporating the conflicting results of other researchers, suggested that the "correct" viscosity of RJ, together with higher consumption is the key factor for queen determination[66].

Thus, today, there is not an accepted unifying hypothesis, which explains how RJ works to produce the bee queen.

A. *mellifera* queen bees differs from the worker bee in several aspects:

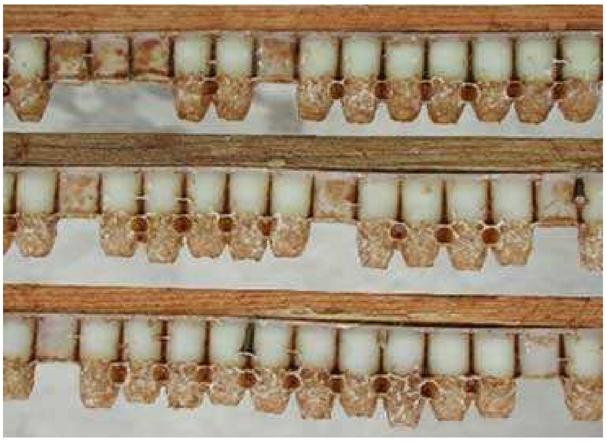
- *Morphology:* Contrary to the workers bees, the queen bee has reproductive organs
- *Development period*: the average queen develops in 15 days, while the worker requires 21 days
- *Life span*: The queen lives for several years, while the worker bees lives several weeks (during summer) to several months (in winter)
- *Behaviour*: The queen lays several thousand eggs a day, while workers lay only occasionally. Unlike workers, the queen does not participate in common hive activities.

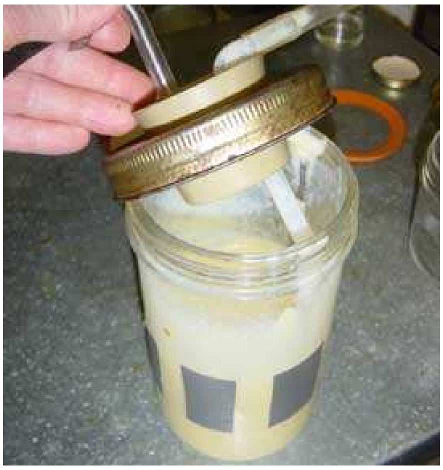
Mainly the fertility and the longer life span of queen bees created the myth of RJ as a wonder-creating food. Although RJ has proven biological effects, it is far from having the status of a food creating similar wonders in humans.

PRODUCTION OF ROYAL JELLY

Royal jelly production steps





Images: 1, 2, 4: Y. Kohl; other images: G. Ratia.

1. Introduce drop of royal jelly in each artificial cell (image 1).

2. Take a young larvae (less than three days old) from a comb and place it in the cell (image 1). Introduce frame in the hive. The bees start raising the queens: Images 2, 3.

3. After 3-4 days the maximum amount of RJ is produced. Take frame with queen cells and uncover the cells. The queen larvae is in a cell full of RJ. About 0.3 g of RJ is in the cell, ready for harvest: Image 4.

4. The RJ is sucked off with a pipette and filtered (filter mesh-size 0.2 mm). Store clean RJ in a cool dark place: Images 5 and 6

It was found that the RJ quality depends on the time of harvesting. Optimum RJ quality is achieved when RJ is harvested 72 hours after grafting of the larvae[85]

Fresh royal jelly

Normally bees produce small quantities of RJ because they nourish a few queen larvae. In order to produce bigger RJ amounts beekeepers use bees‘ instinct to produce a new queen, if the old one is away. RJ is produced in specialised beekeeping units, where up to 500 g per colony can be produced in one season. Two production methods are used:

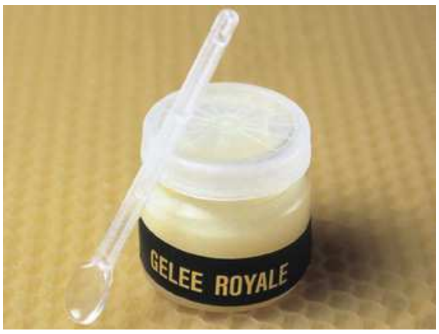
- **Discontinuous** method: Take away queen from the colony and introduce a frame with artificial queen cells containing larvae. This frame will be exchanged every 3 days, while the RJ is harvested. After 3-4 times a harvest break should be made as the RJ quantity diminishes strongly.
- **Continuous** method: This method allows a permanent production during the whole bee season. A nucleus, composed of the queen, together with a small part of the colony is placed near the parent colony. After every second RJ harvest the combs of the nucleus and the queenless mother colony are exchanged, so that the queen has more room for laying and new nursing bees and larvae can be transferred from the nucleus to the queenless colony.

Presently the continuous method is mostly used for commercial RJ production.

The production steps are the same for both methods.

According to the book “Apiculture in China [17] the main factors for optimum RJ production are:

- Breeding of high RJ producing bee strains
- Use of strong colonies
- Optimum number of cells per colony. In general about 1 g of royal jelly is produced from 3 to 4 comb cells.
- Adjust the cycle royal jelly extraction and use brood of the right age. If royal jelly is extracted every 3 day the brood should be 1 to 1.5 days old. If the brood is extracted once every other day the brood should be 2 to 2.5 days old
- Provide enough feeding and water for the colonies (pollen, nectar). If no enough pollen is available, feed with pollen substitutes. The production of RJ should not be interrupted unless there is emergency
- When RJ is extracted the brood must not be broken when the queen cells are con or the brood is picked out. After extraction RJ should be filtered with a 100 mesh nylon net and store at –18 C^0 .

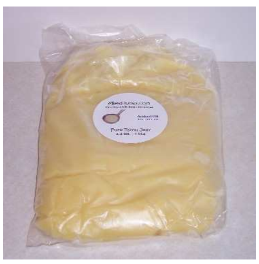


Details of RJ production are described in recent publications [15, 16, 19, 26, 47-49, 80]. A system has been developed at Zhejiang University to produce high yields of royal jelly from a new strain of honey bees by optimising the different production steps [16] According to this method the RJ production methods could be improved by better queen selection, new equipment, prolongation of the production period, improved manipulations skills, technology and feeding. Presently, about 150 g per colony over 3 days can be produced, and 7.7 kg per colony and year[16].

A.m.ligustica and *A.m.caucasica* are better suited for RJ production than *A.m.mellifera* [25, 29].

Feeding of the colony during the productions period of the colony should be optimum with sugar syrop or sugar patties with pollen, but no pollen replacement nutrients should be used [25].

An economic production of royal jelly and its rapid conservation method have been proposed [29].



Freeze-dried royal jelly

Freeze-dried royal jelly is a very hygroscopic powder. It is obtained by evaporating the water content from the frozen product in vacuum. This is the drying process which best maintains the original characteristics of the product: it retains the volatile components which would be removed by evaporation at higher temperatures and does not damage nor denature the thermolabile components.

Freeze-drying requires special equipment, ranging from a simple laboratory freeze-drier to large industrial machines. Though the small laboratory models are normally used for analysis only, small volumes of royal jelly can be processed adequately with this size of equipment.

For drying, the royal jelly is first diluted with some clean water. This leads to a more regular and complete loss of water, particularly if large quantities are freeze dried in one batch. No such preparation is necessary if royal jelly is dried directly in the sales vial. During the final drying phase, in order to achieve more complete removal of residual water, the substrate can be warmed very slightly, but never above 35 ^{0}C.

After freeze-drying, the royal jelly becomes extremely hygroscopic and must be protected from the humidity of the environment by storage in an air-tight container. Larger processors handle freeze-dried royal jelly only in controlled atmospheres, i.e. air conditioned rooms with very low humidity. Depending on the final use of the dried royal jelly, a carrier base or stabilizer can be added at this point to reduce the hygroscopicity of the dried product, e.g. cyclodextrin [78].

Freeze-dried royal jelly marketed directly to the consumer is usually presented in separate vials one or more for a liquid solvent and others containing the dry phase. This is the best solution for conservation without chemical preservatives. The liquid phase can be pasteurized and packed aseptically, without damaging the heat sensitive royal jelly. Freeze dried RJ has the same biological properties as fresh one[59]

STORAGE AND SHELF LIFE

Storage

Freshness has been attributed a great importance for RJ quality. Royal jelly can be spoiled easily if not properly stored. Immediately after harvest it should be placed in dark vessel and stored 0 - 5°C. Stored under these conditions its quality remains OK for half an year. Deterioration of royal jelly can be prevented by storing RJ in Argon after harvesting[28]. After longer storage it will turn rancid. Frozen royal jelly can be lyophilised as it can be transported more easily in the dry state. If frozen, it can be stored for 2-3 years without loosing of its quality. Chauvin states that the physical properties of RJ change after 20 hours after harvest, if left at ambient temperature [14]. That means that RJ should be stored in the cold immediately after harvest. According to Chauvin RJ the biological properties of RJ in what regards its capability to induce hyperglycaemia, remain intact only for 1 month, if stored at about 4°C. On the other hand Krylov tested whole RJ, stored for one year at 5 °C and found out that its antimyocard activity, was not different, in comparison to fresh RJ[37]. Recently it was also shown that only storage of RJ in frozen state prevents decomposition of biologically active RJ proteins[50].

On the other hand, storage experiments of fresh RJ and FTIR measurements of protein degradation showed that after 21 months of storage at -20°C the protein begins to decompose. When RJ is stored at 4 °C RJ should be stored for a maximum of 7 weeks[76]

Experiments have shown that the enzyme glucose oxidase enzyme contained in RJ is influenced both by storage temperature and time[2, 6]. At 4°C there was small reduction of enzyme activity, while at 20°C it decreases significantly after one month and degrades completely after one year[6]. At 37 and 50°C this decrease is faster[3]. The determination of glucose oxidase is analytically very simple and thus within the capabilities of all laboratories. This method could be used to evaluate the product's freshness; however, further investigation must first be conducted into the natural variability of this component in the fresh product.

Recently it was proposed that furosine content can be used as a marker for RJ freshness[53]. The initial content of this compound is very low in fresh royal jelly. Specifically, the content rose to as high as 500 mg/100g of protein after 18 months' storage at room temperature and 50 mg/100g at 4°C. Samples taken from store shelves showed values ranging from 40 to 100 mg/100g protein. The value of furosine, a product of Maillard's reaction, proved very low (from 0 to 10 mg/100g of protein) in freshly produced RJ samples but increases over time and in relation to temperature. A limit of 50 mg furosine / 100g protein could be used for fresh RJ. A specific RJ protein, decomposing upon storage can also be used as a freshness marker[30]. A cheap and fast method based on a chromogenic reaction of RJ and HCl has been proposed[86].

Improvement of storability

From the above findings it is clear that RJ is an unstable product. Freeze drying is the most important technological method in order to improve the storability of RJ. However, there is a loss of volatile substances, as reported by Vahonina, 1995 in [10]. Stabilisation can be achieved by mixing 1 to 2 % of RJ into honey, where all enzymatic activity is stopped.

As reported in[10] the Russian Braines found out in 1968, that RJ can be bound to a mixture of lactose and glucose, which improves its durability. In Russia RJ is often offered in such lactose-glucose pills under the name of Apilac. The method of Braines was improved as follows: Six part of frozen RJ are added to one part of dried glucose-lactose (1:1), then the mixture containing 50 mg/kg L-ascorbic acid as an antixodant is dried until 4 % humidity. This product is stable at 4 to 8 ^{0}C for 2 years[9, 10].

COMPOSITION

Royal jelly is a viscous jelly substance. It is partially soluble in water with a density of 1.1 g/mL. Its colour is whitish to yellow, the yellow colour increasing upon storage. Its odour is sour and pungent, the taste being sour and sweet. The sensory characteristic is an important quality criterion. Old royal jelly, which has not been properly stored tends to be darker and a rancid taste can develop. For optimum quality it should be stored in frozen state. The viscosity varies according to water content and age - it slowly becomes more viscous when stored at room temperature or in a refrigerator at 50 °C. The increased viscosity appears to be related to an increase in water insoluble nitrogenous compounds, together with a reduction in soluble nitrogen and free amino acids [75]. These changes are apparently due to continued enzymatic activities and interaction between the lipid and protein fractions.

There is no international standard for royal jelly, while some countries like Brazil, Bulgaria, Japan, Switzerland and Uruguay have established national ones. A working group of the International Honey Commission is working on the elaboration of an international standard. A first work in view of establishment of a standard has been published[64]

Composition of royal jelly after[64]

	Fresh	lyophilized
Water %	60 - 70	< 5
Lipids %	3 - 8	8 – 19
10-hydroxy-2-decenoic acid %	> 1,4	> 3,5
Protein %	9 – 18	27 – 41
Fructose + glucose+ sucrose %	7 – 18	
Fructose %	3 – 13	
Glucose %	4 – 8	
Sucrose %	0,5 – 2,0	
Ash %	0,8 – 3,0	2 – 5
pH	3,4 – 4,5	3,4 – 4,5
Acidity (ml 0.1N NaOH/g)	3,0 – 6,0	

Humidity

The water content, with 60-70 % is the main component of royal jelly. The dry substance is composed of carbohydrates, proteins, amino acids and fat. Smaller quantities of minerals and vitamins are also present (see table).

Proteins and peptides

With 17 to 45 % of the RJ dry weight they are the main substance class of RJ [39], and also the principal nitrogenous compounds, accounting for about 97-98 % of them [38]. About 60 % of them are water-soluble [38].

The structure and function of the nine most abundant proteins, Major Royal Jelly Protein (MRJP) has been reviewed in 2014. They have most probably a nutritional function for feeding the young larvae and play also a role for honeybee development and colony organisation[11]. MRJP1 is a freshness marker of RJ quality[72].

Apisin, a protein that is unique to royal jelly (RJ), is known to compose the greater part of the RJ proteins and to exist as a heterooligomer containing MRJP1 and apisimin. The apisin content was fairly constant (3.93 to 4.67 w/w%) in natural RJ. A simple, standardized method for quantifying apisin using HPLC is described , which suggests that apisin can be used as a benchmark for future evaluations of RJ quality[20]

Free amino acids represent only 0.6 - 1.5% , the majority of which belong to the L series. The most representative are proline and lysine [7, 68]. Upon storage at 4°C for 10 months no significant changes of amino acids were encountered, while after room temperature storage proline and lysine content increased [7]. This is due probably to proteolytic enzyme activity.

A number of enzymes have been characterised: glucose oxidase [2, 65], invertase [2, 77], acid and alkaline phosphatase, alpha and beta esterase, leucine aminopeptiadase,valin aminopeptiadase lipase, phosphoamidase, [77] and superoxide dismutase [33].

Lipids

The lipids with 3 to 19 % of the RJ dry weight [7, 41], are second in importance after the proteins. 80 to 90 % of the lipid fraction consists of free fatty acids, the rest being neutral lipids, sterols, hydrocarbons [34, 39, 41, 43, 44].

Most of the organic acids are free with rather unusual structure rarely encountered in nature, mono- and dihydroxy acids and dicarboxylic acids with 8 and 10 carbon atoms [40, 41]. The identification of this fraction – in particular as regards the pattern and quantitative analysis of free organic acids – is believed to represent the criteria of choice for defining the genuineness of RJ [6, 12]. The main acid 10-hydroxy-2-decenoic (HDA) is an unsaturated acid, which is determined for the evaluation of RJ genuinely (see Quality parameters and Standard)

HDA and also the other fatty acids of RJ have antibacterial properties [56, 70], thus contributing to the relatively low content of bacteria in this product.

The other fatty acids are all saturated mono- and dihydroxy-, mono- and dicarboxylic acids have not been quantified exactly, but can be roughly estimated to be around 0.5 to to 1 g/ 100 g [43]

Carbohydrates

These are third in importance, composed of mainly fructose, glucose and sucrose [42, 45, 69], with some traces of maltose, trehalose, melibiose, ribose and erlose also being found [42, 45].

Minerals

The ash content (minerals) represents 0.8 to 3 % of RJ fresh matter.

The major elements are K, P, S, Na, Ca, Al, Mg, Zn, Fe, Cu and Mn but there are trace amounts (0.01-1 mg/100 g) of Ni, Cr, Sn, W, Sb,Ti and Bi. The sodium content of RJ varies between 11 and 14 mg/ 100 g. [74]

Vitamins

The concentrations of vitamins in RJ are distributed over a broad spectrum; vitamins showing fairly uniform values are riboflavin, thiamine, niacin and folic acid. Likewise present but with greater variations are pyridoxine, biotin, pantothenic acid and inositol.

Only traces of vitamin C are present, while the fat soluble vitamins like vitamine A,D, E and K are absent [67].

Other minor components

Numerous minor compounds, belonging to diverse chemical categories, have been identified in royal jelly. Among these are two heterocyclic substances, biopterine and neopterine at 25 and 5 µg/g of fresh weight respectively [62]. These compounds are found in the food of worker bee larvae too, but at about one tenth of these concentration. Other substances identified include several nucleotides as free bases (adenosine, uridine, guanosine, iridin and cytidine) the phosphates AMP, ADP, and ATP [54], acetylcholine (1 mg/g dry weight, [23] and gluconic acid (1.4 % of fresh weight, [58]. Benzoic acid (8-15 mg/kg) has also been found [55]. Small amounts of malic, lactic and citric acid have also been found [32]. RJ has antioxidant activity[60] for which polyphenols are responsible[52]

QUALITY AND STANDARD

There is no international standard. Some countries as Brazil, Bulgaria, Japan, Switzerland and Uruguay have national standards [64]

Sensory requirements

Sensory test	Requirements
Colour	White to yellow, yellow colour increases with storage
Odour	Sour, pungent
Taste	Sour, sweet
Consistency	A viscous jelly
Visual purity	Pollen, very few wax and larvae particles

Quality requirements for royal jelly and standards[64]

Parameter	Requirement		
	Fresh RJ	**Lyophylized RJ**	**Reference, analysis**
General quality			
Humidity	Max. 70 g/100g	Max. 5 g/100g	[71]
10-HDA (HPLC)	> 1,9 g / 100 g	> 3,5 g / 100 g	[13, 31, 35]
Furosine (HPLC,GC-MS)	Max 50 mg / 100 g*	Max 50 mg / 100 g*	[53, 57, 81]
Purity, Authenticity	Corresponds to requirements, see [5, 64]		

A possible adulterant of royal jelly is bee brood. Bee brood has a similar composition as RJ (see bee brood section) as to the type of components, but their concentration is different, allowing the proof of bee brood adulterated RJ.

The authenticity of royal jelly is a major quality issue.

Generally, 10-HDA is used to test authenticity, however this substance can be manipulated by adding it to RJ. HDA The apisin content was fairly constant (3.93 to 4.67 w/w%) in natural RJ. A simple, standardized method for quantifying apisin using HPLC is described , which suggests that apisin can be used as a benchmark for future evaluations of RJ quality[20]

There are other methods for the determination of the authenticity of RJ[64, 82, 83]: the most recent being a combination of sugar analysis and stable isotope ratio mass spectrometry to detect the use of artificial sugars in royal jelly, [82, 83]

Irradiation of RJ which can deteriorate RJ quality can be traced by Electron Spin Resonance spectral analysis[84]

Royal jelly can be contaminated by antibiotics by improper beekeeping practices[5]. Best quality of royal jelly can be achieved in certified organic beekeeping.

LABELLING

Composition

Fresh, lyophilised

Indicate protein-, carbohydrate and fat content, 10 g RJ correspond to 30 calories

Intake (See Report on health claims, intake)

One full tea spoon of fresh royal jelly is approx. 10 g, determine dosage of RJ on spatula

Fresh RJ: adults: 100 – 250 mg per day; children: half dose

Warning: It is recommended that people who are susceptible to allergies or asthma should avoid intake of royal jelly

Shelf life

Fresh royal jelly:

- 6 months, if stored in the refrigerator (3 to 5 ºC)
- 2 years if stored in the freezer (< - 18 ºC)

Lyophylised royal jelly

- One year if stored in the refrigerator (3 to 5 ºC)
- At least 2 years if stored in the freezer (< - 18 ºC)

Fresh or lyophylised royal jelly in honey

- Two years at room temperature, if honey-RJ total humidity is less than 18 %.

TRADE

Royal Jelly is product, which is very well known in East Asia, while it is much less known in other parts of the world. There are no official statistics on RJ trade. Some figures are given by Crane. In 1984 the annual production was about 700 t. In the same year Japan produced 46 tons of RJ. According to these figures China and Taiwan account for approximately 60 % and 20 % of the world production, the rest is produced in Korea, Japan, Eastern Europe, Italy, France[18]

In America Mexico is the largest RJ producer [36]. In Europe RJ is produced mainly in Eastern Europe, Italy and France. According to a recent article published in 2009 about 3000 tons of RJ are produced annually in China[51] . Thus, today about 4-5000 tons annually are produced world-wide.

In the sixties the whole sale price of RJ was 180 to 400 $ per kg [36]. In 1993 the wholesale price of Chinese royal jelly per kg was 50-80 $, in 2017 it is around 20 to 40 $ for normal RJ, and 30 to 100 $ for organic RJ according to offers in Internet. Thus, there has been an enormous price decline for the main RJ on the market. Prices for European RJ are considerably higher.

According to http://www.mielinfrance.fr/miel-et-apiculture/le-prix-de-la-gelee-royale/ accessed in April 2017 the price of French RJ was 10 euros for 10 g or about about 50 times higher than that of Chinese RJ. Only 5 % of the totally consumed RJ originates from French producers.

Efforts are necessary to insure RJ quality and achieve higher prices which are by all means deserved by such a valuable product.

BEE BROOD

Bee brood (BB) which is the least recognised bee product in terms of use for human nutrition. Drone brood (DB), instead of bee brood should be harvested in order to keep bee populations stable.

In earlier cultures this product was probably of second greatest importance after honey. Bee brood could therefore serve as a direct food source once the beekeeper has no more need for extra bees or brood, or when undesired colonies have to be removed. Honeybee brood of all ages is eagerly consumed by honey hunters in Africa and Asia and is generally considered a delicious treat. The brood is said to form a considerable part of their diet (Hill et al., 1984 and Bailey, 1989; as cited in [67]). In China and Japan, drone larvae are canned for export or, after being covered in chocolate. DB is eaten most often together with the combs or the pupae can be picked out and pickled or boiled. Indeed, bee brood is particularly rich in protein.

In 1960 it was estimated that 132 tons of bee brood was destroyed before winter just in the Canadian provinces of Alberta, Manitoba and Sasketchewan. They wanted to develop a market for this food and found out that the most accepted form was deep fat frying of the brood. When brood was prepared by either shallow frying in butter or deep-fat frying in vegetable cooking fat and tested by a panel of Canadians, "Most reactions were favourable and some were eulogistic; initial prejudice proved easier to overcome than we had expected. When the tasters were asked to compare the material to some more familiar food, those most commonly mentioned were walnuts, pork crackling, sunflower seeds, and rice crispies. In a later, larger taste test, deep-fat fried, butter fried, and baked preparations were highly rated while smoked, pickled, and brandied were much less preferred[24]

In many Asian and African countries fresh DB is considered a delicacy. A bee brood products named “bakuti”, is produced in Nepal, described in 1990 by Burgett: Sections of comb are placed in a woven, fabric bag and hand squeezed over an open container that collects the liquid phase. This is then heated and gently stirred until it becomes the consistency of soft scrambled eggs. The odor and flavor of bakuti, Dr. Burgett describes as "nut like." To make it more acceptable to the U.S. palate, he mixes an equal volume of Philadelphia brand cream cheese and serves the preparation on crackers. In Zimbabwe the Shona use three kinds of hive, recognized as mukuyo (honeycombs), the machinda (bee pupae), and the pfuma (royal jelly). "Only the mukuyo honey is taken home, that from the machinda hive is either eaten on the spot or thrown away and that from the pfuma eaten there and then." A cake-like mass made from honey boiled with millet, and called chihungwe, is eaten as a delicacy or may be taken to other villages and sold or bartered for grain [8]

BB is regularly sold alongside honey in markets in many parts of Asia [67].

Harvesting

Different aspects of harvesting bee brood as food were investigated. To insure uniformity of larval age at harvest time, brood rearing was concentrated in certain frames by confining queens in frames having queen excluder walls. Every fourth day the comb filled with eggs was removed from the cage and replaced by an empty brood comb. Brood was allowed to develop until most of the larvae were capped (9-11 days). Cells can be uncapped with a thin serrated knife, and larvae are extracted easily and efficiently by spraying the comb with one or more jets of water. Larvae are removed from both sides of the comb and allowed to fall onto a cloth filter such as cheesecloth. After the water is shaken from the cells, the dark empty brood combs can be returned to the queens. The queens prefer them and they encourage maximum egg production. The author states that it is possible to harvest at least one pound of larvae per week from each producing queen[21]

In a 2016 publication another harvesting method is described, with considerably more details:

Honey bee colonies

"*Honey bee brood can be harvested from the colonies at different stages in the development from larvae to adult bee. To maximize biomass of the harvested brood it should not be harvested before the larvae stop feeding, which is at the time of capping. As pupae grow older, there will be an increasing amount of chitin (from the yellow thorax stage in Figure 2) making the brood less palatable. Just before emergence the brood resembles adults with considerable amounts of indigestible chitinous cuticle and has an aversive taste. In addition, the pupae will lose weight during the pupation period. Based on these considerations, brood should be harvested before the pupae eyes becomes pink, corresponding to 0–130 h and 0–160 h post-capping for worker and drone brood respectively. Harvesting of DB, which has to be taken away from bee colonies in the frame of alternative Varroa control is a good occasion for harvesting.*

Harvesting in the field and processing

To avoid contamination of toxic chemicals brood should not be harvested from colonies treated with toxic substances used for protection against pests and parasites.

To reduce the risk of microbiological contamination harvested brood combs should be kept in clean hive boxes or other suitable containers that allows transportation without physical damages to the brood. Both open and capped brood will stay alive at room temperature for a few hours allowing for transport from apiaries to facilities where the brood can be processed or stored. If brood frames are cut out from a frame in the apiary additional care should be given due to the risk of nutritious liquid leaking from damaged brood. Therefore each brood piece should be placed in clean plastic bags or containers and kept cold during transportation to facilities where the brood can be processed or stored. The brood should be frozen as soon as possible after harvest, at −20 °C ideally within 4–6 h after harvest to ensure the freshness of the product.

Work in clean environments with clean hands, gloves and tools. Work only with one comb piece at a time and let the rest be in the freezer. Break the comb piece into small pieces with hands in the large (~3 l) container. Remove individual brood from the wax with forceps or tweezers into smaller plastic containers. Every 5 min, before the frozen brood begins to thaw, place the small container of separated brood back into the freezer and pull another small container from the freezer. Repeat, processing comb from the freezer one piece at a time. Rear small containers once the separated brood is re-frozen and keep in the freezer before using for new separated brood.

Pour liquid nitrogen into an insulated food-grade container. Drop pieces of brood comb (~100 cm2) into the container. Let it freeze completely (about 20–30 s). Use a spoon or a steel strainer to lift the chunk of comb out of the liquid nitrogen and place onto a clean plastic tray. Quickly rub and break up the brood comb using gloved hands to separate all the frozen wax and debris from the brood. Rub the brood in between a dry cloth to remove any remaining debris.

Rubbing should be gentle in particular for pupae not to cause breakages between the thorax and abdomen. Once clean, place the brood in a small container and store in a freezer.

Brood juice"

Use unfrozen or thawed brood combs. Place a stainless steel sieve (⌀ = 18 cm) above a plastic container (⌀ = 20 cm). Squeeze comb pieces above the sieve and let the juices pass the sieve. The juice needs to be frozen or used quickly"[27]

Apilarnil is a Romanian product, based on drone bee larvae and its food. Its composition is similar to the one of royal jelly [73] but outside Romania there is no published scientific data on this product.

Composition

The composition of Bulgarian bee brood and royal jelly after[4] is given in the table below:

Mean±SD and ranges (n=7)	Royal Jelly	Drone brood
Water content, %	63.39±1.75 (61.00-65.20)	70.97±0.72 (70.30-72.30
Protein, %	16.73±1.29 (14.65-18.33)	9.35±0.63 (8.12-10.00)
Fructose, %	4.88±0.37 (4.24-5.35)	0.11±0.11 (0.00-0.34
Glucose, %	3.46±0.58 (2.70-4.15)	6.74±0.65 (5.92-7.88)
Sucrose, %	1.53±0.55 (0.59-2.05)	0.05±0.07 (0.00-0.18)
Total sugars, %	9.86±0.93 (8.47-10.80)	6.92±0.70 (6.22-8.22)
pH	3.95±0.09 (3.80-4.02)	6.49±0.14 (6.23-6.63)
Total acidity, ml 0.1 N NaOH	4.07±0.30 (3.68-4.42)	0.88±0.15 (0.74-1.10)
Electrical conductivity, uS/cm	205.14±8.73 (194.00-219.00)	161.43±10.67 (144.00-178.00)

There are no quality requirements for bee brood, as it is generally not sold on the market.

Storage and shelf life

This topic was studied in detail by Burimistrova[10]. The conclusions of the studies are:

- The maximum storage of fresh drone brood (FDB) at room temperature is one hour, then it has to be cooled or frozen
- The maximum storage at 4 to 8 ^{0}C is 12 to 24 hours
- At -20 ^{0}C it can be stored until 3 months

For better storage six parts FDB it is added to one part of dried glucose-lactose (1:1) the mixture containing 50 mg/kg L-ascorbic acid as an antioxidant is dried until 4 to % humidity. This product is stable at 4 to 8 ^{0}C for 2 years.

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