

BEE DRONE BROOD HOMOGENATE CHEMICAL COMPOSITION, STABILIZATION AND APPLICATION: A REVIEW

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Abstract

The aim of this study was to review research findings and information about chemical composition and application of bee drone brood homogenate for food purposes. As the world's population grows, global warming and the impact of meat production on the ecosystem are increasingly being discussed. Various non-traditional sources of protein, such as insects and larvae, could replace traditional sources of meat protein in the future. Drone brood homogenate is obtained from honey bee drone larvae and is considered to be a very high value by-product of beekeeping. Scientific studies prove the prophylactic properties of drone brood homogenate to improve fertility and strengthen immunity against viral diseases. This product is rich in nutrients, amino acids, vitamins, minerals and hormones but a certain harvesting and processing technology is required to ensure that the product has a sufficient shelf life and an attractive appearance.

Key words: drone larvae, *Apilarnil*, honeybee products, bee pupae, brood homogenate, drone brood, drone milk.

Introduction

Honey and other bee products have been known for a very long time. These products are used as food and food supplements to improve health, because honey and other bee products are deemed to be prophylactic (Çelik *et al.*, 2019). Modern beekeeping is inconceivable without the presence of the *Varroa* mite (*Varroa destructor*). This parasite is a global problem for beekeepers, as it can lead to the death of a bee colony. Bees collect pollen and pollinate plants. Pollination is necessary for the plants to reproduce. The death of a bee colony affects the reproduction of plants, which is necessary for the maintenance of the ecosystem (Flores *et al.*, 2021). Today, the topical issue is the change in the use of varroasis control products, as *Varroa* mite in the world have become accustomed to synthetic pesticides, and bee colonies are weakening due to the increasing use of chemicals in agriculture (Kasiotis *et al.*, 2021). Many chemicals are banned in the European Union because they have a negative impact on the ecosystem. Harmful substances must not be allowed to enter beekeeping products. One of the most gentle ways to combat the spread of *Varroa* mites and other diseases is to remove bee drone larvae from the hive (Charrière *et al.*, 2003). Beekeepers on organic farms use this technique to control *Varroa* mites, because bee drone cells are attracted by a significantly higher number of mites than working bee cells. The development cycle of bee drone brood is longer than the development of working bees; therefore, *Varroa* mites develop in drone brood cells, because mites in working bee broods do not reach the maturity necessary for the production of offspring. Bee drone cells are 1.5 times larger in diameter than working bee cells (Yadav, Kumar, & Jat, 2017). The second reason why bee drone larvae are removed from the hive is to limit the number of drones for working bees. Bee drones eat a

significant amount of honey from the hive and cause losses of honey to beekeepers. Thus, bee drone brood homogenate is produced from drone larvae as a by-product of beekeeping.

Drone brood homogenate (DBH) is one of the bee products also known as *Apilarnil* discovered by Romanian beekeeper Mr. Nicolae V. Ilesiu. It is a homogeneous milky product with a sour taste, yellowish colour or sometimes a shade of grey (Barnutiu *et al.*, 2013; Sawczuk, Karpinska, & Milytk, 2019). The product is obtained by removing bee drone larvae from hives before closing the cells. In many literature sources, this product has been compared with royal jelly (Bogdanov, 2016; Çelik *et al.*, 2019; Sawczuk, Karpinska, & Milytk, 2019), which is milky secretion produced by worker honeybees for the purpose of feeding larvae and adult bee queens (Pavel *et al.*, 2011).

Bee drone larvae are rich in nutrients, vitamins and hormones. The aim of this study was to review the research findings and latest information about chemical composition and application of bee drone brood homogenate for food purposes.

Materials and Methods

The monographic method was used to summarise the research findings and latest information about bee drone brood homogenate for food purposes. Information was searched for in Science Direct, Scopus, Web of Science, Google Scholar. The following keywords were used in the selection of scientific literature: 'drone larvae', '*Apilarnil*', 'honeybee products', 'bee pupae', 'drone brood', 'drone milk' and 'brood homogenate'.

Results and Discussion

Harvesting of bee drone larva

Bees have different stages of development: egg, larva, pupa and imago (adult stage). Drones, the males

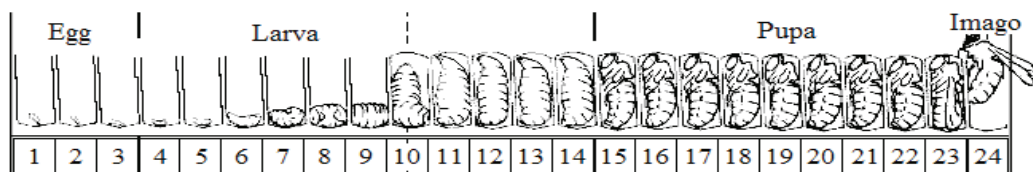


Figure 1. Stages of bee drone development (Modified by the authors, source: Development of the honey bees, 2021).

of the colony, are produced from unfertilized eggs. The developmental period of drones is 24 days (Yadav, Kumar, & Jat, 2017). From days 1 to 3, there is an egg (Figure 1). The size of a bee egg is approximately 1.5 mm (Woyke, 1998). From day 4 to day 14, there is the larva. From day 15 to day 23, there is the pupa. On day 24, an imago is formed from the pupa. On day 10, the working bees cap the larval cells of the drones. Bee drone larval harvesting should be planned at a certain stage in the life cycle of the bee drone. Day 10, when the drone cell is capped, it could be used to determine the age of the larva.

The day of the developmental cycle when it is harvested has a significant effect on larval composition. In the Romanian patent No. 0043842 B1 (Iliesiu, 1984), it is mentioned that bee drone larvae should be collected on day 10 of the development cycle. In patent RU No. 2402920 (Луцук, Дьяченко, & Белик, 2010), the larvae should be 9–10 days old. The stage of larva is characterised by a high nutritive value. The stage of prepupae begins from day 10 to day 14. In Patent US 2014/0323.014 A1, it is mentioned that the external feature of harvesting is when the prepupae are in a vertical position in the capped cells, with colourless or slightly yellowing eyelets without pigmentation (Trifonov *et al.*, 2014). The harvested larvae are then homogenized and filtered. Species, region, climatic conditions and flora are likely to have significant effects (Sawczuk, Karpinska, & Miltik, 2019).

Characteristics of drone brood homogenate chemical composition

Scientific studies provide information on protein levels depending on the age of the larvae (Grishina & Gengin, 2016). There is a tendency that as the age of the larva increases, the ratio of protein to larval

mass increases. The trend of increasing protein concentration depending on the stage of development can also be found in other scientific research (Ghosh, Jung, & Meyer-Rochow, 2016). The working bee’s pupa contains more protein than the larva, but the amount of carbohydrates decreases (Table 1).

Characteristics of DBH are presented in the Table 2, which shows the chemical composition for both fresh and lyophilised DBH and royal jelly. The composition of DBH is similar to royal jelly. Therefore, these products are often compared in research on bee products (Bogdanov, 2016). It is shown that the content of fructose and sucrose DBH is lower than in royal jelly. The chemical composition of DBH varies in different studies, but is within certain limits.

DBH was defined as a perspective alternative **protein** source if appropriate production, collection and processing is organized (Ulmer, Smetana, & Heinz, 2020). Geographical location and climate affect the composition of both royal jelly and larvae. The bee species could also have some effect. There are at least eight honeybee species in the world (Bees4life, 2021). European honeybee (*Apis mellifera*) is the most common worldwide. This specie is native to Europe, western Asia, and Africa. Proteins supplied in a lyophilised format are more stable for storage purposes. Potential degradation pathways for proteins: hydrolysis; oxidation; racemization; isomerization; denaturation; adsorption. Lyophilised proteins should not be exposed to atmospheric oxygen. Higher level than pH 8 should also be avoided. In pharmacy, it is recommended that lyophilised peptides and proteins should be stored at temperatures –20 °C or –80 °C to minimize degradation (Manning *et al.*, 2010).

Table 1

Approximate nutrients composition of larva, pupa and imago (adult) development stage, % of dry matter (Ghosh, Jung, & Meyer-Rochow, 2016)

Nutrients	Larva	Pupa	Imago (adult)
Protein	35.3	45.9	51
Fat	14.5	16	6.9
Carbohydrate	46.1	34.3	30.6
Ash	4.1	3.8	11.5

Table 2

Characteristics of fresh and lyophilised drone brood homogenate and royal jelly

Characteristics*	Drone brood homogenate		Royal jelly	
	fresh	lyophilised	fresh	lyophilised
pH	4.80–6.70	-	3.40–4.50	-
Acidity, ml 0.1N NaOH g ⁻¹	0.88–3.28	-	3.00–6.00	-
Water, %	65.0–76.4	3.0–4.5	60.0–70.0	2.7
Protein, %	4.6–12.0	32.0–52.4	9.0–18.0	27.0–41.0
Lipids, %	1.3–8.0	21.1–24.2	3.0–8.0	8.0–19.0
Carbohydrates, %	6.0–12.2	17.8–38.9	7.0–18.0	26.8
Ash, %	0.7–2.0	2.7–4.1	0.8–3.0	3.5
Energy value, kJ 100 g ⁻¹	503.3	1974.8–2097.9	381**	1525.9

‘-’ – information not found; *Data collected from Barnutiu *et al.*, 2013; Bogdanov, 2016; Isidorov, Bakier, & Stocki, 2016; Margaoan *et al.*, 2017; Sawczuk, Karpinska, & Milytk, 2019; Silici, 2019. **Calculated values.

Table 3

Comparison of hormone content in prepupae, bee drone brood and royal jelly

Hormones, nmol (100 g) ⁻¹	Prepupae with yellowing eyelets (10–14 days old)*	Fresh drone brood (9–11 days old)**	Fresh royal jelly**
Testosterone	0.292–0.322	0.310±0.015	0.200 ± 0.030
Progesterone	42.63–60.01	51.32 ± 8.69	4.61 ± 0.26
Prolactin	344.60–475.40	410.00 ± 65.40	70.80 ± 20.00
Estradiol (oestradiol)	431.20–847.90	677.60 ± 170.30	52.00 ± 6.00

*Data obtained from Trifonov *et al.*, 2014; Трифонов *et al.*, 2013; **Data obtained from Bogdanov, 2016.

Bee drone larvae contain much higher concentrations of **hormones** than royal jelly (Table 3). In Patent US 2014/0323.014 A1 it is mentioned that drone prepupae with yellowing eyelets guarantee hormone concentrations within certain limits (Trifonov *et al.*, 2014).

DBH can be used as a hormone preparation to improve fertility due to the significant hormone concentration of this product. Testosterone is the primary androgen responsible for the development and maintenance of male sexual characteristics. Progesterone is the dominant ovarian hormone with the function to prepare the uterus for embryo implantation. Prolactin stimulates the breasts to produce milk. Estradiol is the dominant ovarian hormone during the follicular (first) phase of the menstrual cycle (BPACNZ, 2013).

Amino acids perform a vital role in the development and maintenance of body. Humans can only synthesize 11 of the 20 important amino acids, which are necessary to make proteins. The other nine essential amino acids are obtained from the diet. Essential amino acids are involved in the biochemical processes of protein synthesis. Amino acid deficiency

impairs immune function and increases the susceptibility to diseases (Kubala, 2018; Wu, 2009). Bee drone larvae contain eight of nine essential amino acids: methionine, histidine, threonine, phenylalanine, isoleucine, valine, lysine, and leucine.

The concentration of amino acids in drone larvae is higher than in worker larvae, thus bee drone larvae are a better source of amino acids (Ghosh *et al.*, 2020; Ghosh, Jung, & Meyer-Rochow, 2016).

Drone brood homogenate is a rich source of **vitamins**. Vitamins are necessary for the normal growth, development and functioning of the body (Yaman *et al.*, 2021). The vitamin content is influenced by the diversity of plants used by bees to feed larvae and produce honey (Silici, 2019). DBH contains large amounts of water-soluble B vitamins, such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acids (B5), pyridoxine (B6), biotin (B7), folic acid (B9), and cobalamin (B12) (Kistanova *et al.*, 2020; Silici, 2019). Vitamin C and choline are also found in DBH (Finke, 2005).

Important **minerals** in DBH are potassium (K), sodium (Na), calcium (Ca), magnesium (Mg) (Özkoç, & Erdem, 2017). A study by Narumi (2004) found

zinc (Zn), iron (Fe), phosphorus (P), manganese (Mn), copper (Cu) and large amounts of selenium (Se) in honeybee brood. Selenium has not been found in other studies. This can be explained by the fact that selenium comes from the soil. Selenium is taken up from the ground by plants, which are pollinated by bees, so that selenium enters bee products (Li *et al.*, 2017; Zhao *et al.*, 2005).

Application of drone brood homogenate

Due to the chemical composition of bee drone larval homogenate, this product is widely used in veterinary medicine (Kistanova *et al.*, 2020; Shoinbayeva *et al.*, 2017), but few studies on the effects on human health have been found (Sidor, & Džugan, 2020). DBH is also discussed in apitherapy (Çelik *et al.*, 2019). Scientific studies prove the prophylactic properties of DBH to improve fertility and strengthen immunity against viral diseases.

Improving semen quality of stud rams (male sheep). Adding the preparation of DBH in the dosages of 15 mg kg⁻¹ of live body weight once per day increased the volume of ejaculate to 30.4%,

concentration of spermatozoa in the ejaculate up to 14.3%, mobility of spermatozoa increases to 9.2 points (Shoinbayeva *et al.*, 2017).

Improving fertility of female pigs. Gilts feed supplementation with the DBH promotes folliculogenesis in the ovaries of gilts at morphological and gene expression level (Kistanova *et al.*, 2020).

Stimulation of genital development. The raw DBH increased the relative organ weights of the glans penis, seminal vesicle and *levator ani* muscle in rats. It also increased the average prostate weight. The raw DBH was able to increase the relative organ weight of the uteri in immature female rats (Seres *et al.*, 2013).

The use of DBH for feeding male broilers (Yucel *et al.*, 2011) did not result in a significant increase in total body weight, but **improving laying efficiency** of hens was detected (Muravev & Kalachinskaya, 2014).

Immunity improvement to prevent viral diseases. Consumption of DBH with vitamin D₃ significantly increased the body's immunity to viral diseases. The patients received 1 tablet of the preparation 2 times a day during a three-month course.

Table 4

Methods for drone brood homogenate stabilisation

Method	Short description of processing parameters	Effect on product quality during its storage	References
Freezing	Brood in the combs, at -20 °C	Without severe change of taste can be stored for 6 months	Jensen <i>et al.</i> , 2019
	Cleaned brood in vacuum-sealed bags, at -20 °C	Without severe change of taste can be stored for 10 months	Jensen <i>et al.</i> , 2019
Freeze drying (lyophilisation)	The lyophilised powder was prepared by freeze drying to the temperature of -5 °C, under residual pressure of 0.03 mmHg for 24 h	The product retained the original biological properties	Shoinbayeva <i>et al.</i> , 2017
	The freezing is done rapidly in temperatures of -50 °C and -80 °C, primary drying at pressure of a few millibars, secondary drying can even be above 0 °C	Lyophilised DBH obtained moisture content of 1% to 4% and retained the original biological properties	Berk, 2013; Bogdanov, 2016
Mixing with honey or sugars	Mixed with honey (not more than 1-2% of brood)	Mixtures can be stored for 6 months at room temperature	Bogdanov, 2016; Бурмистрова <i>et al.</i> , 2008
	Adsorption on glucose-lactose (1:1) and dried until 4% moisture content	Can be stored at 4-6 °C up to 2 years	Bogdanov, 2016; Бурмистрова <i>et al.</i> , 2008
	Mixed with lactose and glucose, then drying is performed with IR-rays during 4 hours at 42-45 °C in the "heating-cooling" pulse mode	Moisture content decrease and biologically active substances preservation, reduction of microbiological contamination and decay prevention	Чекрыга, Волончук, & Левин, 2012
Adding ethanol	DBH combined with 40% and 70% ethyl alcohol with added antioxidant	Obtained stable tincture which has an extended shelf life and retains its biological activity of the components for 2 years	Будникова, 2011; Гашиш <i>et al.</i> , 2018

There were no cases of acute respiratory disease or influenza in the patients during the autumn and winter period (Strukov *et al.*, 2016).

Processing and preservation of drone brood

Drone larvae should be harvested together with the combs. Removal from the hive should be done carefully so as not to injure the larvae. Both open and capped larvae will stay alive at room temperature for a few hours after removal from hive (Jensen *et al.*, 2019). Other studies suggest that larvae should be refrigerated immediately after cutting out from the combs. Freezing, drying, boiling or frying should be completed less than 24 hours after collection of larvae to avoid any spoilage. Insect proteins decay much faster than those of beef, pork, lamb or chicken (Bogdanov, 2011; Krell, 1996). Rapid larval deterioration may begin after homogenisation, thus DBH requires immediate processing under strict hygiene requirements. One of the solutions used by beekeepers is to freeze DBH at -20 °C or mix it with honey in certain proportions (Hroshovy *et al.*, 2021; Isidorov, Bakier, & Stocki, 2016). Sugar helps to prevent or slow down the growth of bacteria, moulds and yeast in food (SNRC, 2021). Honey, sucrose, lactose or glucose can be used as preservatives (Митрофанов *et al.*, 2015). Different stabilization technologies can be used to efficiently extend the shelf life of DBH (Table 4).

Freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapour without passing through a liquid phase (FDA, 2014). The absence of water prevents microbial growth and chemical changes leading to deterioration of product. Lyophilised DBH kept the original biological properties, measured by its immunomodulating, spleen and T-cell stimulating properties (Bogdanov, 2016). Freeze drying did not reduce DBH hormone levels (Sidor & Džugan, 2020). The maximum allowable drying temperature must not exceed 45 °C, as this will inactivate the hormones and biological activity in the product (Будникова, 2011; Корж, 2009). Freeze drying could be the most suitable drying technology for DBH to improve the shelf life of the product. Spray drying could also be used, but elevated temperatures can degrade product quality - the product loses hormones and vitamins.

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Most of the consumers avoid eating insects, larvae or worms, which could be one of the alternative sources of protein in the future (Higa *et al.*, 2020). Encapsulated DBH would be more attractive and convenient for consumers.

DBH is a product rich in nutrients, minerals and hormones, and its effective use is possible through certain harvesting and processing technologies. The shelf life can be extended by freezing and drying, and additives can be added. The processing technology used must not impair the valuable composition of the product. The resulting product must be acceptable and safe for the consumers. In the future, DBH could be widely produced as a food supplement in Latvia as well, but extensive research is needed on the processing and chemical composition of DBH obtained in the Baltic region and its impact on human health and safety.

Conclusions

Drone brood homogenate (DBH) is considered to be a very high value by-product of beekeeping, which can bring additional income to beekeepers if it is properly processed. Geographical location, climate, species and stage of development affect both the composition of the royal jelly and the larvae. Bees feed drone larvae with royal jelly, but DBH is made from drone larvae, so the composition of these two beekeeping products is similar, but DBH has elevated levels of sex hormones. In order for DBH to obtain the highest hormone concentration, the collection of drone larvae should be performed on days 10 to 14 of the developmental stage, observing the external feature: the prepupae in a vertical position in the capped cells, with colourless or slightly yellowing eyelets without pigmentation. Scientific studies show that the use of DBH has health benefits, including improved fertility of animals, strengthening immunity to prevent viral diseases. DBH is a product rich in nutrients, amino acids, vitamins, minerals and hormones but a certain harvesting and processing technology is required to ensure that the product has a sufficient shelf life and an attractive appearance. DBH was defined as a perspective alternative protein source, which could replace pork or beef in the future.

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