

INCREASING THE QUANTITY AND QUALITY OF ROYAL JELLY IN APIARIES USING WAX HONEYCOMB CUPS

Lazat Umiraliyeva^{1*}, Marat Amangeldin¹, Akniet Ibraikhan¹,
Timur Krupskiy², Alina Tulegenova²

¹Kazakh Scientific Research Institute of Processing and Food Industry,
Gagarin Avenue 238, 050060 Almaty, Republic of Kazakhstan

²Kazakh Scientific Research Institute of Animal Husbandry and Forage Production,
Zhandosova 51, 055552 Almaty, Republic of Kazakhstan

*e-mail: l.umiraliyeva@rpf.kz

Abstract

Royal jelly production is a vital aspect of apiculture, yet achieving high yields while maintaining the health of bee colonies remains a challenge in industrial apiaries. This study addresses the need to enhance royal jelly production without compromising the production of other beekeeping products. The aim of this research was to compare the most effective technology for the formation of a nursery family for obtaining royal jelly in the field and to compare the queen cell acceptance by showing the type of material used in our experiment. The study outlines a comprehensive technology for obtaining royal jelly, encompassing key operations such as cup preparation, larval inoculation, colony preparation, jelly collection, and transportation. Emphasis is placed on the fundamental principles of royal jelly production in an industrial setting, with particular attention to stimulating queen cell formation and intensive larval rearing.

Two variants of nursery family formation were investigated: one with complete orphanage and the other without orphanhood.

The non-orphanhood method exhibited superior results, boasting a 33% larval adoption rate and 25% more mature queen cells compared to the complete orphanhood approach. Furthermore, this method yielded larger mother liquors and maintained colony productivity without interruption, addressing key concerns in royal jelly production.

This study presents an innovative approach to royal jelly production that prioritizes both yield and colony

health. The non-orphanhood method, coupled with the use of wax cups and silicone molds for larval transfer, offers a promising solution to enhance royal jelly production on an industrial scale, while preserving its biological value.

Key words: *Beekeeping, Royal jelly, Honeycomb cups, Larvae grafting.*

1. Introduction

Royal jelly (RJ) is biosynthesized along with wax and venom directly by honeybees as a hive product. It is secreted by the laryngeal and mandibular glands of young worker bees at the age of 5 to 14 days and serves to indiscriminately feed all larvae during the first 3 days of their life. Only larvae selected to become queens continue to feed on RJ throughout their life cycle [1]. The assumption of this functional food stimulates the phenotypic development of the female bee larva, allowing it to develop into a fertile queen bee instead of a sterile worker bee. The consumption of RJ can additionally significantly prolong the life cycle of bees [2].

RJ can be consumed by humans as a nutritional integrator and has a high commercial value due to its nutritional and nutraceutical properties. In fact, it is rich in various nutrients beneficial to humans, including proteins, sugars, vitamins, and a large number of biologically active substances such as 10-hydroxy-2-decenoic acid (10-HDA) [6]. The 10-HDA content is widely regarded as a most important parameter

of RJ quality evaluation, e.g. a minimum of 1.4% is specified in the international standard of RJ [8]. Royal jelly is used as a health food and natural cosmetics in many countries [7]. It is one of the most profitable bee products, as the price per gram is much higher than that of honey or pollen [1 - 7].

Many colonies of bees are managed using traditional beekeeping methods [9]. Traditional beehives are basic cylindrical containers in which bees and their combs are housed. They are hives with permanent honeycombs that are typically housed in hollow logs or clay or Wicken vessels. Traditional beekeeping does not use contemporary equipment or practices [10]. However, in recent years, the well-known type of movable frame hive, the modern hive (MH), has been introduced and is now actively advocated. It is a modern beekeeping technique that provides an alternative system for frame hives [11]. The main claimed benefits of a moveable frame hive are that it is significantly more productive and easier to manage bee colonies than a regular hive.

The commercial production of RJ is based on the characteristics of providing RJ in bee colonies. RJ is synthesized and secreted mainly by the hypopharyngeal and mandibular glands of nurse bees to feed queen bees throughout their lives and worker larvae only during the first three days [11, 12]. Thus, RJ determines whether the female larva develops into a reproductive queen or a sterile worker one [12]. At the larval stage, nurse bees deposit a greater amount of RJ in the queen cells in which queen bees are grown compared to working cells [12].

RJ can be produced commercially as its market value is higher than other bee products, including honey, pollen and propolis. However, RJ has become the main source of income for beekeepers worldwide [12]. For example, China is one of the largest producers and exporters of RJ, harvesting 4,000 tons annually, representing over 90% of the world's RJ production. It is mainly exported to the USA, Europe, and Japan [12]. Some other countries and regions, including Vietnam, Taiwan, Korea, and Japan, are important manufacturers and exporters of RJ. It is also produced in Eastern and Western Europe such as Greece, Spain, France and Italy, as well as in Mexico [12].

Various biotic and abiotic factors influence the quantity and quality of RJ. The most important factors are races of honeybees, colony type, i.e. queen less or queen, age of larval transplantation, number of transplanted queen cells [12], grafting methods, level grafting bars and queen cell position, harvesting interval, food source, and seasons. For example, the feeding of bees with sugar syrup causes significant changes

in the amount and structure of vital RJ components, such as amino acids, carbohydrates, and vitamins [12]. Apiculture scientists are making significant efforts to develop various tools, grafting methods to select highly productive honeybee strains to increase RJ production.

The present study was conducted to compare the most effective technology for the formation of a nursery family for obtaining royal jelly in the field and to compare the queen cell acceptance by showing the type of material used in our experiment.

2. Materials and Methods

The technology for obtaining royal jelly includes the following operations: preparation of cups, inoculation of larvae into cups, preparation and use of nursery family, collection of royal jelly and its preparation for transportation.

The basic principles of obtaining royal jelly in industrial production with artificial breeding of queens: the bee colony, left without a queen, immediately starts growing new, young queens. However, stimulation of the formation of queen cells and intensive rearing of larvae in them with royal jelly in mass quantities has a number of features.

When considering the advantages of one or another method of forming nursery families in industrial apiaries, it is necessary to evaluate all components of beekeeping technology, given that the production of royal jelly should not reduce or worsen the production of other beekeeping products.

We have developed a technology for obtaining royal jelly, it includes the following operations:

1. Preparation of grafting frames. A certain number of manufactured wax cups are attached to the grafting frames;
2. Preparing the bee colony for royal jelly. During the month, the bees are fed with specific food, 0.5 liters every other day;
3. Formation of nursery family and collection of royal jelly;
4. Collection of royal jelly, storage and transportation.

Combs with coeval larvae were obtained using a frame insulator from a split grid. The honeycomb was installed in the laboratory on a special stand in an inclined position. The larvae were removed from the cells with a spatula from the dorsal side together with a small amount of royal jelly and transferred to prepared cups, placing them on the bottom or on a drop of pre-poured food so that the position of the larva in the bowl did not change, that is, it corresponded to its position

in the cell of the comb. By inserting the slats with cups and larvae into the frame, if the slats are removable, or by turning the rotating slats with cups and larvae by 90°, a finished grafting frame was obtained, which contains about 60 larvae. Immediately after the end of the inoculation of the larvae, the inoculation frame was placed in the host colony.

Royal jelly is obtained subject to the temperature regime within 25 - 30 °C and humidity not more than 70%. With a spatula, the queen larvae are taken out of the cups, and the remaining royal jelly is taken out. Then the process of obtaining queen cells is repeated, after which the cells of the queen cells are again attached to the grafting frames and placed in the hives of the nurse families.

One of the main problems in the technology of royal jelly production is the choice of the most effective way to form nursery family.

Two variants of the formation of a nursery family were tested: with complete orphanage and without orphanhood (Table 1).

Table 1. Influence of ways of formation of nursery family on production royal jelly

Index	Method of formation of a nursery family	
	With complete orphanage	Without orphanage
Selected royal jelly from one mother liquor, g	0.3 ± 0.027	0.42 ± 0.019
Selected royal jelly from the bee colony, g	6.5 ± 0.9	8.7 ± 0.4
Duration of family use	no more than 30 days	55 - 60 days or more
Number of adopted larvae for rearing	48	72

As a result of the research, it was found that the method of forming a nursery family without orphanage showed the best results: the number of adopted larvae for rearing was 33%, and the number of mature queen cells was 25% more than with complete orphanhood.

The mother liquors grown in the nursery family, formed by the method without orphanage were much larger than with complete orphanage. In addition, when forming a host colony in a way without orphanage, the host colony did not stop working on honey collection and pollen collection, and there were no fears that the bee colony might become rotten.

When collecting queen cells, the weight of royal jelly obtained in a family without orphanhood was significantly higher. This technology is being tested on a pilot apiary in the East Kazakhstan region in nomadic

apiaries, as it may well become the basis for obtaining royal jelly on an industrial scale.

In the production of royal jelly, the research team used cups made from an organic material, beeswax (Figure 1). Both light and air have a bad effect on royal jelly, and after only 15 minutes after being extracted from the mother liquor, it loses most of its beneficial and healing properties. Even if the beekeeper is experienced and knows how to properly carry out the extraction process, he will not be able to get a large amount of royal jelly, since it must be extracted from literally every single cell.



Figure 1. Silicone molds for casting wax bowls and wax bowls

We have proposed a method for storing royal jelly in queen cells without extracting it from it. At the same time, it retains all its biological values.

For the manufacture of wax cups, wooden templates 8 - 10 cm long with a rounded polished end 8.5 - 9 mm in diameter are used by hand, which are immersed in cold water 30 minutes before work, then lowered 4 - 5 times into melted in a water bath (wax temperature about 70 °C) wax (preferably drip wax) by 7 - 8 mm, each time reducing the immersion depth so that the base of the bowl is thicker than its walls. The finished bowl was cooled in water and removed from the template by rotating it. To prepare wax cups, we used the method of casting wax cups into silicone molds (Figure 1). This innovative method makes it possible to obtain a large number of cups for the transfer of larvae in a large volume and at the right time.

3. Results and Discussion

The most effective technology for the formation of a nursery family for obtaining royal jelly in the field for nomadic apiaries has been developed - the formation of a nursery family in a hive bed without orphanage. The number of adopted larvae for rearing was 36% more than in the first method (with complete orphanage) and 18% more than in the second (with partial orphanage). The mother liquors grown in the nursery family formed by the third method were much larger than in the first and second cases.

The existing methods of production and processing of native royal jelly have been improved and tested using gentle processing methods (freezing, adsorption). In the first method, queen cells with milk taken from the nursery family were placed in a freezer for storage in order to further study their quality. In the second method, native royal jelly was adsorbed with a mixture of lactose and glucose to obtain a dry powder with a residual moisture content of 5%.

Acknowledgement

The research was supported by the financing of the scientific and technical program of the Ministry of Agriculture of the Republic of Kazakhstan for 2021-2023 BR10764970 "Development of high-tech technologies for deep processing of agricultural raw materials in order to expand the range and output of finished products from a unit of raw materials, as well as reduce the share of waste in production" within the framework of the project "Development of technology for the production and processing of royal jelly for the food industry". The team of authors expresses sincere gratitude to all participants of this scientific project for their help and assistance in conducting research. We also express our gratitude to the management and scientists of the Kazakh Research Institute of Processing and Food Industry for their assistance and support.

4. Conclusions

- As a result of the study, it was found that the second method, i.e. the formation of a nursery family without orphanage showed the best results: the number of adopted larvae for rearing was 20% more than in the first method. Also, mature mother liquors were obtained by the second method by 13% more than in the first one.
- The mother liquors grown in the nursery family formed by the second method were much larger than in the first and second cases. In addition, in the second method, the nursery colony did not stop working on honey collection and pollen collection, and there were no fears that the bee colony might become rotten.

Thus, the developed technology for the production of royal jelly in the formation of a nursery family without orphanhood can be used in the field for use in industrial production.

- To breed queens and collect royal jelly, the whole world uses cups made of inorganic material, which are strong pollutants for the world ecosystem and cause damage to the production of royal jelly, since bees do not accept them and the mortality of larvae increases. We have developed an organic production method based on the same bee product, which is the most optimal method for the production of royal jelly. The bees accept cups made of beeswax at 100%, and the survival rate of larvae on such cups has approached the maximum. The method is being tested in an experimental apiary for filing a patent for inventions.

5. References

- [1] Kunugi H., Ali A. M. (2019). *Royal jelly and its components promote healthy aging and longevity: from animal models to humans*. Int. J. Mol. Sci., 20, (4662), pp. 1-26.
- [2] Ramadan M. F., Al-Ghamdi A. (2012). *Bioactive compounds and health-promoting properties of royal jelly: A review*. J. Funct. Food, 4, pp. 39-52.
- [3] Wytrychowski M., Chenavas S., Daniele G., Casabianca H., Batteau M., Guibert S., Brion B. (2013). *Physicochemical characterisation of French royal jelly: comparison with commercial royal jellies and royal jellies produced through artificial bee-feeding*. J. Food Compos. Anal., 29, pp. 126-133.
- [4] Takenaka T., Takenaka Y. (1996). *Royal jelly from Apis cerana japonica and Apis mellifera*. Biosci. Biotech. Bioch., 60, (3), pp. 518-520.
- [5] Garcia-Amoedo L. H., Almeida-Muradian L. B. (2007). *Physicochemical composition of pure and adulterated royal jelly*. Quím Nova, 30, (2), pp. 257-259.
- [6] Viuda-Martos M., Ruiz-Navajas Y., Fernández-López J., Pérez-Álvarez J. A. (2008). *Functional properties of honey, propolis, and royal jelly*. J. Food Sci., 73, pp. 117-124.
- [7] Pasupuleti V. R., Sammugam L., Ramesh N., Gan S. H. (2017). *Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits*. Oxid. Med. Cell Long., 1259510. DOI:10.1155/2017/1259510. Accessed 12 June 2022.
- [8] ISO. (2016). *ISO 12824 - Royal jelly: Specifications*. BSI Standards Publication, 12824, pp. 44.
- [9] Gratzer K., Wakjira K., Fiedler S., Brodschneider R. (2021). *Challenges and perspectives for beekeeping in Ethiopia. A review*. Agronomy for Sustainable Development, 41, (4), pp. 1-15.
- [10] Mulatu A., Marisennayya S., Bojago E. (2021). *Adoption of modern hive beekeeping technology: The case of Kacha-Birra Woreda, Kembata Tembaro Zone, Southern Ethiopia*. Advances in Agriculture. <URL:https://doi.org/10.1155/2021/4714020. Accessed 12 June 2022.
- [11] Serda B., Zewudu T., Dereje M., Aman M. (2015). *Beekeeping practices, production potential and challenges of bee keeping among beekeepers in Haramaya District, Eastern Ethiopia*. Journal of Veterinary Science and Technology, 6, pp. 1-5.

- [12] Wright G. A., Nicolson S. W., Shafir S. (2018). *Nutritional physiology and ecology of honey bees*. Annu. Rev. Entomol., 63, pp. 327-344.
- [13] Hu F. L., Bilikova K., Casabianca H., Daniele G., Espindola F. S., Feng M., Guan C., Han B., Krakova T. K., Li J. K., Li L., Li X. A., Simuth J., Wu L. M., Wu Y. Q., Xue X. F., Xue Y. B., Yamaguchi K., Zeng Z. J., Zheng H. Q., Zhou J. H. (2019). *Standard methods for Apis mellifera royal jelly research*. J. Apicult. Res., 58, pp. 1-68.
- [14] Slater G. P., Yocum G. D., Bowsher J. H. (2020). Diet quantity influences caste determination in honeybees (*Apis mellifera*). Proc. R. Soc. B-Biol. Sci. <URL:https://doi.org/10.1098/rspb.2020.0614. Accessed 12 June 2022.
- [15] Ghramh H. A., Khan K. A., Ahmed Z., Ansari M. J. (2020). *Quality evaluation of Saudi honey harvested from the Asir province by using high-performance liquid chromatography (HPLC)*. Saudi J. Biol. Sci., 27, (8), pp. 2097-2105.
- [16] Altaye S. Z., Meng L., Li J. (2019). *Molecular insights into the enhanced performance of royal jelly secretion by a stock of honeybee (Apis mellifera ligustica) selected for increasing royal jelly production*. Apidologie, 50, (4), pp. 436-453.
- [17] Kanelis D., Tananaki C., Liolios V., Dimou M., Goras G., Rodopoulou M. A., Karazafiris E., Thrasyvoulou A. (2015). *A suggestion for royal jelly specifications*. Arh. Hig. Rada Toksikol., 66, (4), pp. 275-284.
- [18] Van Toor R., Littlejohn R. (1994). *Evaluation of hive management techniques in production of royal jelly by honey bees (Apis mellifera) in New Zealand*. J. Apic. Res., 33, (3), pp. 160-166.
- [19] Shi J. I., Liao C. H., Wang Z. I., Wu X. B. (2018). *Effect of royal jelly on longevity and memory-related traits of Apis mellifera workers*. J. Asia-Pacif. Entomol., 21, (4), pp. 1430-1433.