UNIVERSITY OF LIFE SCIENCES
"KING MIHAI I" FROM Timisoara
Multidisciplinary Conference on Sustainable Development
21 – 22 May 2026

Microbiological Profile and Safety Evaluation of Apilarnil from the South Region of Romania under Different Processing Conditions

Roxana Antoaneta SPULBER[1], Adina Gafițuc[1], Bogdan TACHE[1], Teodora COLȚA[1], Cristina VĂDUVA[1], Maria DINCĂ[1]
Institute for Beekeeping Research and Development, 42 Ficusului Blvd, 013975, Bucharest-1, Romania, e-mail: roxana.spulber@icdapicultura.ro
*Corresponding author: adina.gafituc@icdapicultura.ro

Abstract: Apilarnil (drone brood product) is a natural bee-derived product obtained from young drone larvae, increasingly recognized for its nutritional and functional properties. As a potential novel food at European level, ensuring its microbiological safety is essential to guarantee consumer health and product quality.
This study aimed to evaluate the microbiological profile and safety of apilarnil under different processing conditions (native, frozen, and lyophilized forms). The analyses included the determination of total viable count (TAMC/NTG), yeasts and molds (TYMC), and the detection of *Escherichia coli*, using standardized methods (ISO standards and European Pharmacopoeia). Additionally, the health status of the source apiaries was assessed through parasitological and bacteriological examinations targeting pathogens of epidemiological importance, according to WOAH guidelines. Mycological investigations were also performed to identify fungal contaminants based on cultural and morphological characteristics.
The results indicated that native apilarnil is not a sterile product, with microbial load influenced by raw material quality and handling conditions. However, freezing and especially lyophilization significantly reduced microbial counts. Despite the absence of sterility, all analyzed samples met acceptable safety criteria, demonstrating that apilarnil is safe for human consumption both in fresh and processed forms, and can be successfully used as a raw material in various food formulations when obtained and handled under appropriate hygienic conditions.

• Introduction

Apilarnil, a bioactive product derived from honeybee drone brood (Fig.1), is increasingly explored for its nutritional and therapeutic potential. Despite these promising applications, its complex biochemical composition and high moisture content make it particularly vulnerable to microbial contamination and rapid degradation if not properly processed. Factors such us temperature exposure, storage conditions, and processing techniques influence not only the survival of microorganisms but also the overall safety and stability of the product. Inadequate control of these variables may lead to the proliferation of bacteria, yeasts, and molds, ultimately affecting both product quality and consumer safety.

At the same time, environmental conditions - especially temperature and humidity, play an important role by influencing bee physiology and the biochemical characteristics of hive derived product. These factors can affect the initial microbial load and the stability of apilarnil during post-harvest processing. Given the growing interest in apilarnil as a functional product, there is a clear need for detailed microbiological assessments under varying processing conditions. This study investigates the microbiological profile of apilarnil collected from the southern region of Romania and evaluates how different processing approaches impact its safety, with the aim of supporting improved handling practices and quality standards.

Fig. 1 Drone larvae in brood cells prior to harvesting

• Material and method

Apilarnil samples were collected from different regions in southern Romania and grouped into three experimental batches. All samples were analyzed for the presence of microorganisms considered relevant hygienic–sanitary indicators, with potential implications for final product contamination.

Batch I (AI): Drone larvae were obtained from the institute' production apiary (Ilfov County) prior to cell capping. Larvae aged 7 days (0.2075-0.2513 g) were selected under in-hive conditions of approximately 33.7°C. Samples were processed immediately and incubated at 30°C for total germ count (TGC/g) determination.

Batch II (ACII, ALII): Samples originated from Tulcea County (southern Romania) and included both frozen raw larvae (ACII) and freeze-dried apilarnil (ALII).

Batch III (ACIII, ALIII): Samples were collected from third-party apiaries (Giurgiu County) and included frozen (ACIII) and freeze-dried (ALIII) forms.

Freeze-dried samples were obtained through mechanical processing (trituation and filtration), followed by lyophilization and storage at −18°C. Microbial load of apilarnil samples from all batches was evaluated using standard methods. Total viable count (TVC) was determined by the pour plate technique after aerobic incubation at 30℃ (SR EN ISO 4833:2014). Yeast and molds were quantified at 25℃ (SR EN ISO 21527:2012). Detection of *Escherichia coli* was performed using membrane filtration according to the European Pharmacopoeia. Samples were aseptically prepared as 1:10 dilutions in tryptic soy broth and pre-enriched (35°C, 18-48 h), followed by selective enrichment in MacConkey broth (43-45°C, 18-24 h). Filtration was carried out using 0.45 µm membranes, which were cultured on selective media (MacConkey agar and chromogenic coliform agar) and incubated at 35-37°C.

All analyses were performed in duplicate.

• Results and discussions

As shown in *Table 1*, freezing and freeze-drying significantly reduced the overall microbial load compared to the raw product (AI). Freeze-drying proved slightly more effective than freezing, leading to a further reduction in viable microorganisms at 30°C.

All analyzed batches (I-III) met satisfactory food safety criteria, with values generally close to the lower acceptable limits (*Tables 1-2*). However, elevated yeast and mold counts were observed, in some cases exceeding recommended limits. This may be attributed to high environmental humidity during the harvesting period, as well as potential contamination during collection and handling under non-controlled conditions.

Table 1. Evaluated parameters and results

Serial no.	Sample origin	Batch number	Product name	Parameter		
				NTG/TAMC/g 3 days at 30°C	D+M/TYMC/g 5-7 days at 25°C	Number of E. coli/g
1.	Ilfov	Batch I, series nr. 230625	AI (Apilarnil nativ-martor)	$3,8x10^5$	-	absent
2.	Tulcea	Batch II, series nr. 100725	$AC_{II(mM)}$	$0,9 x10^4$	$4,5 x10^3$	absent
			AL_{II}	$0,7 x10^4$	$3 x10^4$	absent
3.	Giurgiu	Batch III, series nr. 100925	$AC_{III (m)}$	$0,5x10^3$	$6 x 10^5$	absent
			$AC_{III(M)}$	$0,5x10^3$	$4 x 10^4$	absent
			AL_{III}	$0,4x10^3$	$5,7 x10^4$	absent

Table 2. Microbiological Criteria for Food Products

Serial no.	Microbiological indicators	Raw Apilarnil results*		Food products **	
1	NTG/ TAMC/g	50 000 ufc/g	$5x10^{-4}$ufc/g	< 100 000 ufc/g	$1x10^5$ ufc/g
2.	D+M/TYMC/g	1000 ufc/g	$1x10^{-3}$ufc/g	< 100 ufc/g	$1x10^2$ ufc/g
3.	E. coli	<10 ufc/g	$1x10^{-1}$ufc/g	<50 ufc/g	<50 ufc/g

*after Popa, 1964
** based on EU Regulations (EC) no.2073/2005 and (EU) 2470/2017

The absence of E. Coli and other intestinal enterococci was observed, supporting the hygienic and microbiological safety of Apilarnil in the context of novel food.

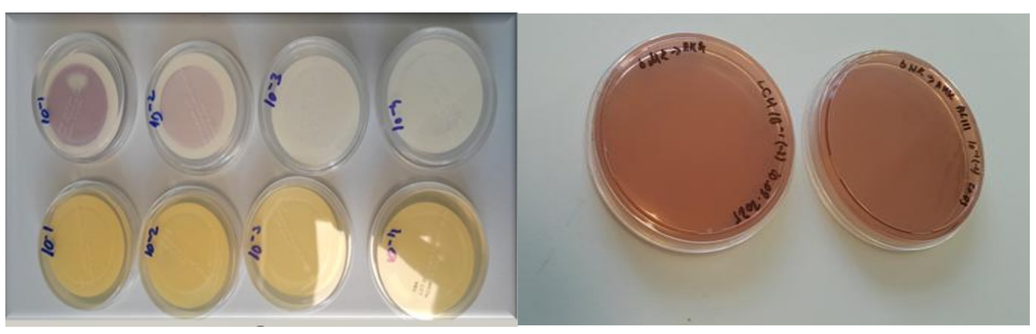

Fig 2. Negative culture results on selective media (MacConKey and chromogenic coliform agar) confirmed the absence of E. coli and other intestinal enterococci.

The most frequently identified micromycetes, based on cultural and morphological characteristics (macroscopic, stereomicroscopic and microscopic examinations), were *Aspergillus clavatus (Fig. 3,4)*, *Alternaris spp*. and *Aspergillus flavus.*

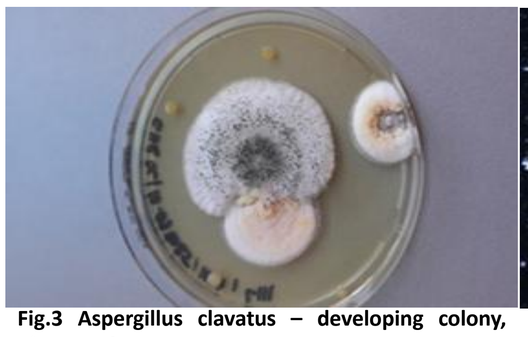

Fig.3 Aspergillus clavatus – developing colony, obverse view

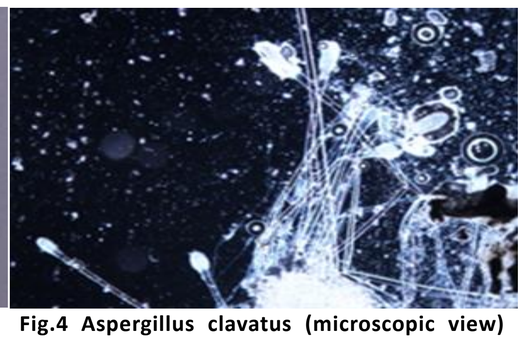

Fig.4 Aspergillus clavatus (microscopic view)

• Conclusions

- Apilarnil is a non-sterile bee-derived product, with microbial load influenced by raw material quality and handling conditions.
- Freezing and especially lyophilization significantly reduced the total microbial load, with freeze-drying showing the highest efficiency.
- All analyzed batches met acceptable microbiological safety criteria, supporting the suitability of apilarnil for human consumption.
- Elevated yeast and mold counts highlight the importance of environmental conditions and hygienic practices during harvesting and processing.
- The persistence of micromycetes such as *Aspergillus clavatus*, *Alternaria* spp., and *Aspergillus flavus* indicates that current preservation methods are not fully effective against fungal spores.
- Apilarnil can be considered a safe and valuable raw material for food applications, provided that appropriate processing and strict hygienic control measures are applied

Acknowledgement: The study was carried out in the frame of national research program ADER 2023-2027, the contract 11.1.2/2023.