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Investigation of microbiological and organoleptic properties of bee products (bee venom, solid pollen, and royal jelly) through water activity quantification during 8 days of storage

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Abstract:	The aim of this investigation was to assess the stability of biological bioregulators for a maximum holding time of 8 days through water activity measurements. Microbiological and organoleptic measurements were carried out in parallel and simultaneously in order to experimentally establish a relationship between the status of the water activity and the microbiological and organoleptic characteristics of the tested bee-derived materials. Apitoxin, solid pollen, and royal jelly were stored for a maximum holding time of 8 days at specific storage conditions such as light-resistance glass containers and under refrigerated storage. For all the bioregulators tested, water activity measurements were performed on days 0, 5, and 8. On days 0 and 8, microbiological and organoleptic assessments were performed. Based on scientific literature, it was established that under these storage conditions, bee venom, solid pollen, and royal jelly exhibited water activity of 0.5278, 0.3088, and 0.9766 respectively, during the entire holding time. The results indicate that water activity can be used as a quality indicator in microbiological and organoleptic stability of raw beekeeping materials intended to be used for the development of new dietary and nutraceutical formulations in different pharmaceutical presentations such as tablets, capsules, lozenges, powder, granulated and oral suspension.

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Editorial board

Journal of Food Science and Technology

Dear sirs:

I am submitting a manuscript entitled "Investigation of microbiological and organoleptic properties of bee products (bee venom, solid pollen, and royal jelly) through water activity quantification during 8 days of storage" by Harold A. Prada-Ramírez, Raquel Gómez Pliego, Sandra Gonzalez-Alarcon, Juan Pablo Montes-Tamara, Romel Peña-Romero, David Díaz-Baez, Gloria Inés Lafaurie, and Humberto Zardo for consideration to be published as an original article in the Journal of Food Science and Technology. All the authors have read and approved the submission of the manuscript to the Journal of Food Science and Technology. As a corresponding author I compromise to review at least three manuscripts about associated topics.

We used a novel validated method called dew point chilled mirror method for water activity measurements as a direct measure of biological burden in raw beekeeping materials such as bee venom, solid pollen, and royal jelly intended to be used as active principal ingredients in nutraceutical, dietary supplements and prebiotic treatments.

Although the dew point chilled mirror method is widely used in tablets, capsules, lozenges, and solid raw, its enforcement is completely unexplored in biological products. In this way, this manuscript provides worthy information about the impact of holding time (samples kept under suitable store conditions) over the microbiological and organoleptic stability through water activity measurements.

The results indicate that water activity can be used as a quality indicator in microbiological and organoleptic stability of raw beekeeping materials intended to be used for the development of new dietary and nutraceutical formulations in different pharmaceutical presentations such as tablets, capsules, lozenges, powder, granulated and oral suspension.

We declare that this manuscript is original, has not previously been published, and is not currently being considered for publication elsewhere. We know of no conflicts of interest associated with this publication, and there has been no financial support for this paper that could have influenced its outcome. As the corresponding author, I confirm that the manuscript has been read and approved for submission by all the named authors.

Conflicts of interest

The authors declare no conflict of interest. All the research was funded by Laboratorios Coaspharma S.A.S.

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Best regards,

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Investigation of microbiological and organoleptic properties of bee products (bee venom, solid pollen, and royal jelly) through water activity quantification during 8 days of storage

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Data Availability Statements

The data underlying this article will be shared on reasonable request to the corresponding author

Ethical Approval Statement

This article has been prepared in accordance with ethical standards and principles. All research conducted for this study was approved by the relevant ethics committee, ensuring that the rights and welfare of participants were safeguarded. Informed consent was obtained from all participants involved in the study, and confidentiality was maintained throughout the research process. The authors declare that there are no conflicts of interest related to this publication.

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All the authors have read and approved the submission of the manuscript to the Journal of Food Science and Technology.

Consent for publication

All the authors give consent for the manuscript to be published, including individual's data or image.

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Not applicable

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ABSTRACT:

The aim of this investigation was to assess the stability of biological bioregulators for a maximum holding time of 8 days through water activity measurements. Microbiological and organoleptic measurements were carried out in parallel and simultaneously in order to experimentally establish a relationship between the status of the water activity and the microbiological and organoleptic characteristics of the tested bee-derived materials.

Apitoxin, solid pollen, and royal jelly were stored for a maximum holding time of 8 days at specific storage conditions such as light-resistance glass containers and under refrigerated storage. For all the bioregulators tested, water activity measurements were performed on days 0, 5, and 8. On days 0 and 8, microbiological and organoleptic assessments were performed. Based on scientific literature, it was established that under these storage conditions, bee venom, solid pollen, and royal jelly exhibited water activity of 0.5278, 0.3088, and 0.9766 respectively, during the entire holding time.

The results indicate that water activity can be used as a quality indicator in microbiological and organoleptic stability of raw beekeeping materials intended to be used for the development of new dietary and nutraceutical formulations in different pharmaceutical presentations such as tablets, capsules, lozenges, powder, granulated and oral suspension.

INTRODUCTION

Aw has been widely used as an indicator of microbiological safety and shelf life in the food industry, because reduction of water activity in foods prevents the growth of vegetative microbial cells, germination of spores, and toxin production by molds and bacteria (1–4). As a matter of fact, Aw is currently used in the food industry as a microbiological quality criterion for product release. There have been several studies carried out at the Coaspharma company that associate the products’ shelf life with Aw levels, since there exists an inversely proportional relationship between durability and water activity (the lower the water activity of a product, the longer it is preserved) (3,4). Therefore, current studies suggest the possibility of using Aw as an indicator of microbiological safety, as described in USP <1112> (1,3). USP chapters <922 and 1112> encourage the pharmaceutical and food industry to use water activity (Aw) as an alternative microbiological method (AMM) for products with low Aw levels, because they are potentially not susceptible to being contaminated (1,2). For instance, solid pharmaceutical forms (powders, lozenges, tablets, and capsules) have had reported water activity around 0.30 to 0.50 which makes them excellent target candidates for excluding microbiological tests lot by lot, because at those low Aw levels, it is unlikely that objectionable pathogens, mesophiles, yeasts, and molds would be able to grow on the pharmaceutical and food articles (3,4). USP chapters 922 and 1112, for instance, recognize new possibilities that allow the implementation of AMMs as Aw measurement as a direct microbiological assessment for the microbiological bioburden determination (1–4). However, Aw must be used as an integral part of routine product release through a risk-based approach that should include Aw measurement, microbiological historical data, chain cold transportation, primary packaging container to prevent oxygen and water vapor exchange, and robust microbiological skip-lot testing each 20 lots or 4 months

(1,3,4). These methods exhibit high performance and the ability to analyze a large number of samples with automated results, allowing real-time analysis and the possibility of early detection of contamination (5–8). Nonetheless, the enforcement of alternative microbiological methods such as Aw measurements remains unexplored in the microbiological and organoleptic assessments of biological products such as bee venom, solid pollen, and royal jelly intended to be used as active principal ingredients in nutraceutical, dietary supplements and prebiotic treatments (9-13).

Over the last decade, an explosion of scientific evidence has proven that beekeeping bioregulators have a wide range of biological attributes that allows mitigating a broad spectrum of diseases such as diabetes, endocrine issues, obesity, inflammation, arthritis, neurodegenerative diseases, multiple sclerosis, kidney issues, amyotrophic lateral sclerosis, thyroid gland disease, wounds, immune problems, and cancer (14–22). Indeed, those bee derivatives have also been shown to be successful for dietary supplement application, and currently they are already included in several dietary and nutraceutical formulations such as tablets, capsules, lozenges, powder, granulated and oral suspension intended as gastrointestinal, pediatric, immune, skin, eye, brain, and oral health (9-13).

Thus, the quantification of Aw is becoming a fundamental tool in the food industry, since it serves to improve decision-making regarding the standardization of storage conditions, process design for ingredients and products, ingredient selection, determining the antimicrobial efficacy of the preservation systems, and finally the selection of primary packaging (1,2).

Holding-time supporting evidence for raw beekeeping materials involves the specified time that a biological sample can be stored prior to manufacturing process without altering the original quality of its attributes (4). Normally, raw materials remain in storage rooms not more than 3 days prior to compounding. Therefore, a maximum holding time of 8 days represents the worst-case scenario to provide evidence that the holding times do not affect raw material quality (Diagram 1). Good manufacturing practices (GMPs) require the necessary measures to be taken to ensure that raw materials are stored under appropriate conditions, so storage conditions should not have adverse effects on the subsequent manufacturing process, stability, safety, efficacy, or quality (4). Holding times must be established to ensure that the bee venom, solid pollen, and royal jelly remain within scientifically established specifications. Regarding that, biological samples may hold an initial bioburden. It is remarkable to test the allowable time by which the beekeeping raw material may be kept under suitable store conditions without negatively impacting quality attributes**.** Thus, the choice of the scientifically supported holding period should be determined by an interdisciplinary team considering the regulatory requirements and manufacturing processes timelines (2,4).

Thus, GMP guidelines state that holding times for dispensed raw materials must be validated to ensure that they do not affect the organoleptic and microbiological characteristics of the initial raw biological material (4).

Experimental Procedures

Materials and Methods

(a) Collection of bee products

Honeybee products such as bee venom, royal jelly, and solid pollen collection were carried out from Huila-Colombia department (southern region) at municipality of Algeciras (2°31′19″ N 75°18′52″ W), during the 2025 summer season. The Huila region is broadly dominated by oak trees and a wide spectrum of flowering plants.

(b) *Storage and Sampling of bee venom, Royal Jelly and Solid Pollen.*

The beekeeping raw materials samples were taken directly from light-resistance screw cap glass containers to proceed with the quantification of the water activity. Beekeeping raw material samples were taken from the top of the containers in order to prevent further handling of the bee derivates. Sterile sampling devices were used. It was ensured that the primary packaging used for the sampling was similar to the original packaging in which the raw material arrived. Light-resistance screw cap glass bottles and high-density polyethylene bags were deemed appropriate for storing bee venom, royal jelly, and solid pollen samples during the holding time assay. Regarding scientific literature high density polyethylene bags are suitable sampling containers because they have a reduced oxygen and water vapor permeability (density 0.945-0.964 g/cm3) (23).

(c) *Reagents*

For the construction of the calibration curve and the determination of the operating range and linearity of the chilled-mirror dew point method, standard saturated salt solutions with known water activity were used. The five standard reference materials used were sourced from METER group, Inc USA, SRM: lithium chloride 13.41 MW +/- 0.5% aw = 0.25, lithium chloride 8.57 MW +/- 0.5% aw = 0.50, sodium chloride 6.0 MW +/- 0.5% aw = 0.76, sodium chloride 2.33 MW +/- 0.5% aw = 0.92, and USP purified water aw = 1.00 +/- 0.003.

The bee derivatives, bee venom, royal jelly, and solid pollen, were supplied by Vital Healthy Solution S.A.S and Laboratorios Coaspharma S.A.S in Bogotá, Colombia.

(d) *Aqualab 4TE*

The equipment used to quantify the water activity was from Aqualab 4TE (METER group), distributed in Colombia by Insulab. The equipment passed the design, installation, operation, and performance qualifications, and the supplier provided training for processing samples and obtaining reproducible results. All this information was documented to comply with user requirements to identify critical parameters for carrying out the methodology (12).

Aqualab 4 TE is an instrument used to quantify water activity via the chilled-mirror dew point method. The system includes a dew point instrument with a precise temperature measurement chamber and Skala Control software to carry out all administrative functions, such as the identification of samples.

Skala Control software stores primary data related to water activity measurements on the Amazon web site (AWS). It complies with CFR 21 Part 11, ensuring the integrity and confidentiality of the data. The system's design qualification (DQ), installation qualification (IQ), operational qualification (OQ), software validation (SV), and performance qualification (PQ) were successfully completed in accordance with PDA guidelines.

(e) *Quantification of Water Activity*

Beekeeping samples of 3 grams were taken on days 0, 5, and 8. These samples were used to quantify the water activity of the beekeeping raw material throughout the maximum holding time of 8 days. The quantification of the Aw was carried out on days 0, 5, and 8 in order to determine the behavior of the Aw during the holding time. On days 0 and 8 of the holding time for each analyzed beekeeping sample, microbiological and organoleptic tests were performed to determine the quality attributes of the raw material.

For Aw measurements, an Aqualab 4TE Water Activity Meter, which belongs to Group B of water activity measuring instruments, was used. For routine water activity measurement, verifications were performed before its use, using acquired standard solutions from METER Group, Inc USA. Four verifications were carried out, with standards of 0.25, 0.50, 0.76, and 0.92, in accordance with the guidelines outlined in Chapter 922 of the USP.

To successfully perform the Aw measurement, a representative amount (1 gram) of bee venom, royal jelly, and solid pollen must be placed into the 15 ml plastic disposable sample container provided by Aqualab supplier, taking into consideration that it should not be above 50% of its overall capacity to avoid mirror chamber contamination during the sample measurement. In the same way, the bee venom, solid pollen, and royal jelly samples should be placed into the 15 ml disposable container, ensuring that the samples completely cover the button of the plastic cup as well as possible, avoiding as much as possible exposure for more than 5 minutes of the samples to the surrounding laboratory environment (minimum 21.6 °C – maximum 34.4 °C and 14.9 %-69.4 % relative humidity) as the sample is placed into the plastic disposable cup and then into the measuring chamber of the dew point instrument. The duration of the whole process usually takes around 50 seconds to take out raw material from the plastic pack. Usually, the Aqualab 4TE takes around 8 minutes to make a reliable Aw measurement.

(f) *Microbiological and Organoleptic Testing*

Microbiological and organoleptic tests were conducted for the samples, taken on days 0 and 8, to determine the microbial load and organoleptic characteristics of the raw beekeeping materials during the holding time. The microbiological analysis included testing for mesophiles, fungi, and yeasts, using conventional methods such as direct plating on agar plates according to USP <61> (24)**.** For all the raw material tested, antimicrobial substances were neutralized to obtain successful microbiological determination. For this, 10 g of the corresponding raw material was added into 90 mL of tryptic soy broth (Merck) SKU No. NCM0004A (dilution 1:10) with tryptic soy broth in the presence of the selected neutralizing agent (1 mL/L of Tween® 80 in tryptic soy broth) and then vigorously shaken for homogenization of the sample. Agar plates (tryptic soy agar and sabouraud dextrose agar) were inoculated in duplicate with 1 mL of this dilution by means of the pour-plate technique. The plates were incubated at 20–25 °C for 7 days for the yeast and mold count and incubated at 30–35 °C for 5 days for the mesophile total count.

The organoleptic testing involved evaluating the appearance, color, and odor of bee derivatives.

Results

Storage and Sampling of bee venom, Royal Jelly and Solid Pollen.

The analyzed raw materials, including apitoxin, royal jelly, and solid pollen, were stored according to scientific literature. As depicted in Table 1, royal jelly and apitoxin were kept under refrigerated storage conditions. Otherwise, solid pollen was appropriately stored at controlled room temperature (20–30 °C). The beekeeping raw materials samples were kept in light-resistance screw cap glass containers to prevent water humidity exchange and sample degradation.

Calibration curve using Saturated salt check standards

As is depicted in Table 2, Calibration curves were constructed from known water activity standard checkpoints in order to confirm that the Aqualab 4TE provides a measurement result that is statistically equivalent to the true value of the Saturated salt check standard solution. In this way, five data points were plotted for the calibration curve (aw = 0.25, aw = 0.50, aw = 0.76, aw = 0.92, and aw =1). For each salt check point six replicates were performed. As it is outlined in USP chapters <922> an acceptance criterion is that the absolute error must be less than the sum of the instrument repeatability and the uncertainty in the standard solution (Table 2). The calibration curves have a validity of one year.

Microbiological and Organoleptic Assessment

Microbiological assessment for apitoxin, solid pollen, and royal jelly were performed using reference standard method based on plate count method for determination of total mesophilic, yeast and mold counts. As is depicted in Table 3, microbiological and organoleptic characteristics of bee venom, propolis, solid pollen, and royal jelly remained within specification during the maximum 8-day holding period (Table 3). Similarly, apitoxin, solid pollen, and royal jelly tested maintained their color, smell, and appearance unaffected, showing the same texture as the original sample (Table 3).

Quantification of Water Activity during the maximum 8-day holding period

As is depicted in Table 4, water activity measurements were performed on days 0, 5, and 8 for bee venom, royal jelly, and solid pollen. For each bee-derivate raw material 6 replicates were performed at days 0, 5, and 8. Average, standard deviation and coefficient of variation were calculated (Table 4). The water activity behavior during the maximum 8-day holding period for bee venom, royal jelly, and solid pollen is depicted in figure 1.

Discussion

The use of Aw as a microbiological, physicochemical, and organoleptic indicator is supported by the current USP (1,2). Indeed, scientific evidence has proven the relationship between Aw levels below 0.75 and microbiological, physicochemical, and organoleptic stability in solid raw materials, lozenges, tablets, and capsules (3,4). The analyzed raw beekeeping materials, namely bee venom, solid pollen, and royal jelly, were stored under controlled storage conditions (Table 1). Furthermore, it was also demonstrated that based on calibration curves using saturated salts that the Aqualab 4TE provides a measurement result that is statistically equivalent to the true value of the Saturated salt check standard solution. In this way, the absolute error was within specification according to USP requirements (Table 2). Stability tests for the 3 raw beekeeping materials analyzed during three different time points for the duration of the testing period showed that the storage conditions for each of them remained constant. The objective of this study was to collect evidence that under the tested storage conditions, the initial microbiological and organoleptic attributes of the bee derivatives were unaffected during the entire holding time (Table 3, Figure 1). Worth mentioning is that solid pollen has an initial bioburden, but it was under acceptable microbiological levels (Table 3). Otherwise, bee venom and solid pollen do not have an initial bioburden.

In this regard, the royal jelly exhibited average Aw levels of 0.9679, 0.9808, and 0.9766 on days 0, 5, and 8 respectively (Table 4). At these water activity levels, royal jelly becomes prone to being spoiled by the activity of microorganisms, since objectionable

Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* and Gram-positive can survive at water activity between 0.95 and 0.97 (1). Bacteria such as *Staphylococcus aureus* can survive at water activity higher than 0.86 (1). On the other hand, yeast and mold can grow at water activity levels greater than 0.75 (1). Nonetheless, as has been previously shown royal jelly contains a natural mixture of different secondary metabolites that are responsible for antimicrobial activity (19, 20).

Otherwise, bee venom and solid pollen exhibited water activity of 0.3088 and 0.5278 respectively during the hold time of 8 days (Table 4). It is well known that at these Aw levels (Aw<0.60), even the most xerophilic fungi and osmophilic yeasts are unable to grow, thus avoiding any contamination of the bee venom and solid pollen (1–4). However, solid pollen has a steady bioburden during the entire holding time (mesophile 120 cfu/g and yeast mold < 60 cfu/g, Table 3). Otherwise, powder bee venom had not shown mesophiles, yeast and molds counts during the entire holding time (< 10 cfu/g, Table 3).

Recent publications put in evidence the preservative potential of bee derivative products, which have excellent coverage against a wide range of microorganisms including bacteria, yeasts, and molds (10,12,13,25). Regarding the low bioburden (<10 cfu/g) found in bee venom and royal jelly, it is possible that some natural antimicrobial agents prevent the proliferation of mesophile yeasts and molds in these bee derivatives (9,10,13). In this regard, bee venom contains a well-known peptide so called melittin which cytolytic potential has been widely proven to provide an intrinsic preservative potential against bacteria, yeast, and fungus (10). Indeed, pure bee venom has been proposed as an alternative to antibiotics in broiler chicken (9). Similarly, several studies indicate that royal jelly acts as a powerful antimicrobial agent because of its bioactive compounds, such as proteins, peptides, phenolic, and fatty acids which have an excellent coverage against bacteria, yeast, and molds (13). In the same way, although solid pollen has an initial bioburden it was steady during the entire holding time assay. It may be explained by antimicrobial properties due to the presence of multiple bioactive compounds (12)

Nonetheless, special attention needs to be paid to royal jelly, because when water activity is > 0.86, the bioregulators might harbor the growth of microorganisms. However, water activity status could be utilized as a useful tool for assessing the microbiological and organoleptic of royal jelly during the holding time trials. For bee venom, solid pollen, and royal jelly, it would be interesting to further assess the preservative potential against several pathogens routinely tested in the pharmaceutical industry, in order to shed light on which microorganisms may potentially contaminate the royal jelly (26).

As depicted in Table 4, the observed uncertainties of Aw for the 6 replicates from the bee venom, solid pollen, and royal jelly exhibited a standard deviation below 0.003 (n=6), showing the high concordance precision of the dew point chilled-mirror method values (Table 4). In order to observe the effect of operational variables such as the effect of different days, a multifactorial analysis of variance was performed (ANOVA $P < 0.05$, Table 4). It is of vital importance to note that for all the analyzed raw beekeeping materials, statistically significant differences in the Aw status were observed between days during the holding time assay (days 0, 5, and 8, ANOVA $P < 0.05$). These variations higher than 0.003 can be considered to be normal, since they may be due to hygroscopic behaviors (tendency to lose or gain water from the environment) of the raw beekeeping materials during the temperature and relative humidity ranges that may occur throughout the day in the storage areas. It is noteworthy to mention that glass containers and the primary packaging used for the sampling (high-density polyethylene bags) prevent oxygen and water vapor permeability, leading to keeps water activity status steady along the maximum holding time of 8 days.

The propensity to gain or lose water from the environment depends on the physicochemical characteristics of each raw beekeeping material and the number and length of time that the drum or barrel is opened during the period of use. For this reason, it is essential to ensure controlled storage conditions and utilize proper primary packaging that steady levels. Furthermore, the instrument used for water activity quantification (dew point chilled-mirror instrument) is the most accurate of the group of instruments for measuring this variable (standard deviation +/-0.003), which results in highly precise measurements. Therefore, variations of the water activity greater than the equipment's' SD during the assay might be statistically significant (Figure 1).

Conclusion

Currently, in the food industry water activity is used as an indicator of microbiological and organoleptic safety and shelf life for a large array of raw materials and products. However, there are no studies regarding the use of water activity as a microbiological assessment of raw beekeeping material such as bee venom, solid pollen, and royal jelly. Thus, this investigation seems to be the first scientific research project that documents the relationship between water activity and microbiological assessment of bee derivatives as an active principal ingredient of nutraceutical and dietary supplements. The results obtained for the raw beekeeping materials bee venom, and royal jelly indicate that the storage conditions (temperature 2-8 ^{o}C and relative humidity 30% - 70%) in the dispensing and raw materials storage areas are appropriate. The results obtained for the solid pollen jelly indicate that the storage conditions (temperature 20-30 ^{o}C and relative humidity 30% - 70%) in the dispensing and raw materials storage areas are appropriate. The organoleptic and microbiological characteristics were not altered during the 8-day waiting period. For bee venom and solid pollen, water activity <0.50 supports holding time of 8 days between dispensing raw material and bulk compounding. Royal jelly with Aw > 0.95, remained stable for the 8-day holding period and evidenced neither organoleptic nor microbiological changes, supporting also the 8-day holing period between raw materials dispensing and bulk compounding.

This study puts in evidence that water activity is a reliable indicator in line of microbiology for bee derivatives. However, we promote actual microbiological and organoleptic tests to be performed as part of vendor qualification and quality audits.

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Table 1. Beekeeping raw materials and their storage conditions during the 8-day holding period.

Raw Material	Storage Conditions
Solid Pollen	Stored in tightly closed glass containers at controlled room temperature (20–30 °C) and 30–70% RH.
Royal Jelly	Stored in closed glass containers at 2–6 °C and 30–70% RH.
Bee Venom	Stored in waterproof, light-resistant glass containers at 2–6 °C and 30–70% RH.

Table 2. Saturated salt check standards used to build up the calibration curves at 25ºC. For each standard, six replicates were taken to calculate average, standard deviation (SD) and relative standard deviation (RSD).

	Calibration curve				
Saturated salt check standards used to build up the calibration curves at 25ºC	**0.25**	**0.5**	**0.76**	**0.92**	**1**
Replicate 1	0.2501	0.5002	0.7601	0.9218	1.0026
Replicate 2	0.2496	0.5006	0.7611	0.9224	0.9999
Replicate 3	0.2498	0.4999	0.7609	0.9224	1.0043
Replicate 4	0.2497	0.4999	0.7609	0.9223	0.9995
Replicate 5	0.2498	0.5	0.7613	0.9234	1.0001
Replicate 6	0.2499	0.4997	0.7607	0.9197	1.001
Average (Aw)	0.2498	0.5001	0.7608	0.9220	1.0012
Standard deviation	0.0002	0.0003	0.0004	0.0012	0.0019
Relative Standard deviation	0.0689	0.0629	0.0543	0.1346	0.1863
Saturated Salt Check Standard (Awº)	0.25	0.5	0.76	0.92	1
Awº (water activity expected) -Aw (water activity experimentally measure)	-0.0002	0	0.0008	0.002	0.0012
Repeatability of the instrument (2*SD)	0.0003	0.0006	0.0008	0.0025	0.0037
Uncertainty of standadrd solution	0.003	0.003	0.003	0.003	0.003
Reproducibility Aqualab + Standard deviation expected according to supplier	0.0033	0.0036	0.0038	0.0055	0.0067
<922> Chapter USP	Absolute error = Awº- Aw ≤ Repeatability of the instrument + Uncertainty of standard solution				

Table 3. Microbiological and organoleptic characteristics of bee venom, propolis, solid pollen, and royal jelly remained within specification during the maximum 8-day holding period.

cfu: colony-forming units.

Raw Material	Organoleptic Characteristics	Total Mesophilic Count	Total Yeast and Mold Count
Solid Pollen	Yellow, viscous jelly with a waxier texture than honey	120 cfu/g	60 cfu/g
Bee Venom	White solid powder (apitoxin)	< 10 cfu/g	< 10 cfu/g
Royal Jelly	Irregular yellow to brown granules; sweet taste	< 10 cfu/ml	< 10 cfu/ml

Table 4. Water activity (aw) measurements of bee venom, solid pollen, and royal jelly on days 0, 5, and 8 of the holding period. Statistical differences in measured parameters for propolis, solid pollen, and royal jelly on days 0, 5, and 8 of the holding period (ANOVA, $P < 0.05$).

		Water activity		
		Day 0 (n = 6)	Day 5 (n = 6)	Day 8 (n = 6)
Solid Pollen	Average	0.27493	0.3115	0.3088
	Standard deviation	0.0007	0.0015	0.0007
	Coefficient variation	0.2376	0.4719	0.2262
Royal Jelly	Average	0.9679	0.9808	0.9766
	Standard deviation	0.0013	0.0019	0.0026
	Coefficient variation	0.1343	0.1912	0.2668
Bee venom	Average	0.4723	0.5395	0.5278
	Standard deviation	0.0009	0.0010	0.0026
	Coefficient variation	0.1965	0.1819	0.4925

Figure 1. Behavior of water activity status for bee venom, solid pollen, and royal jelly during the 8-day waiting period.

Diagram 1. Bee venom, solid pollen, and royal jelly were stored for a maximum holding time of 8 days at specific storage conditions such as light-resistance glass containers and under refrigerated storage. For all the bioregulators tested, water activity measurements were performed using the dew point chilled-mirror method on days 0, 5, and 8. On days 0 and 8, microbiological and organoleptic assessments were performed.

Figure

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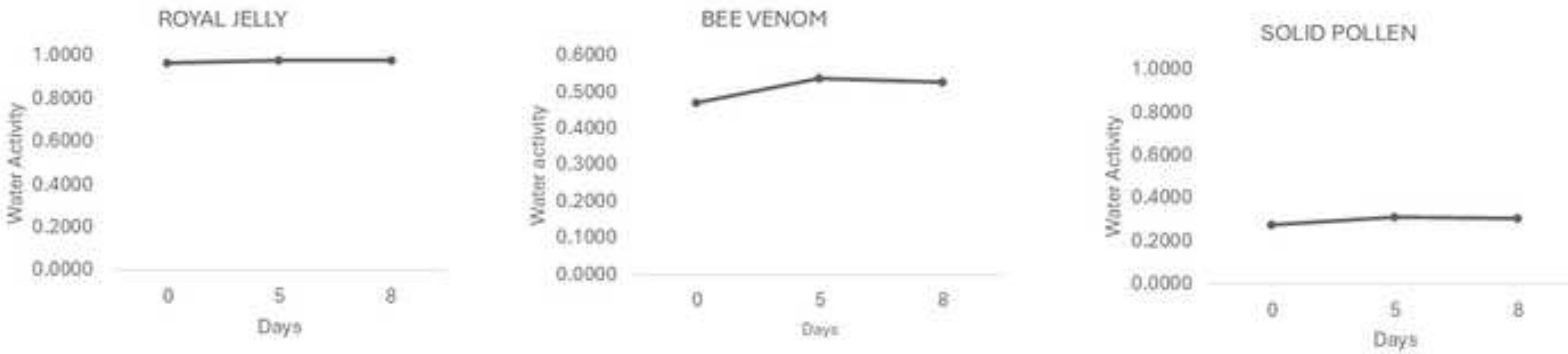

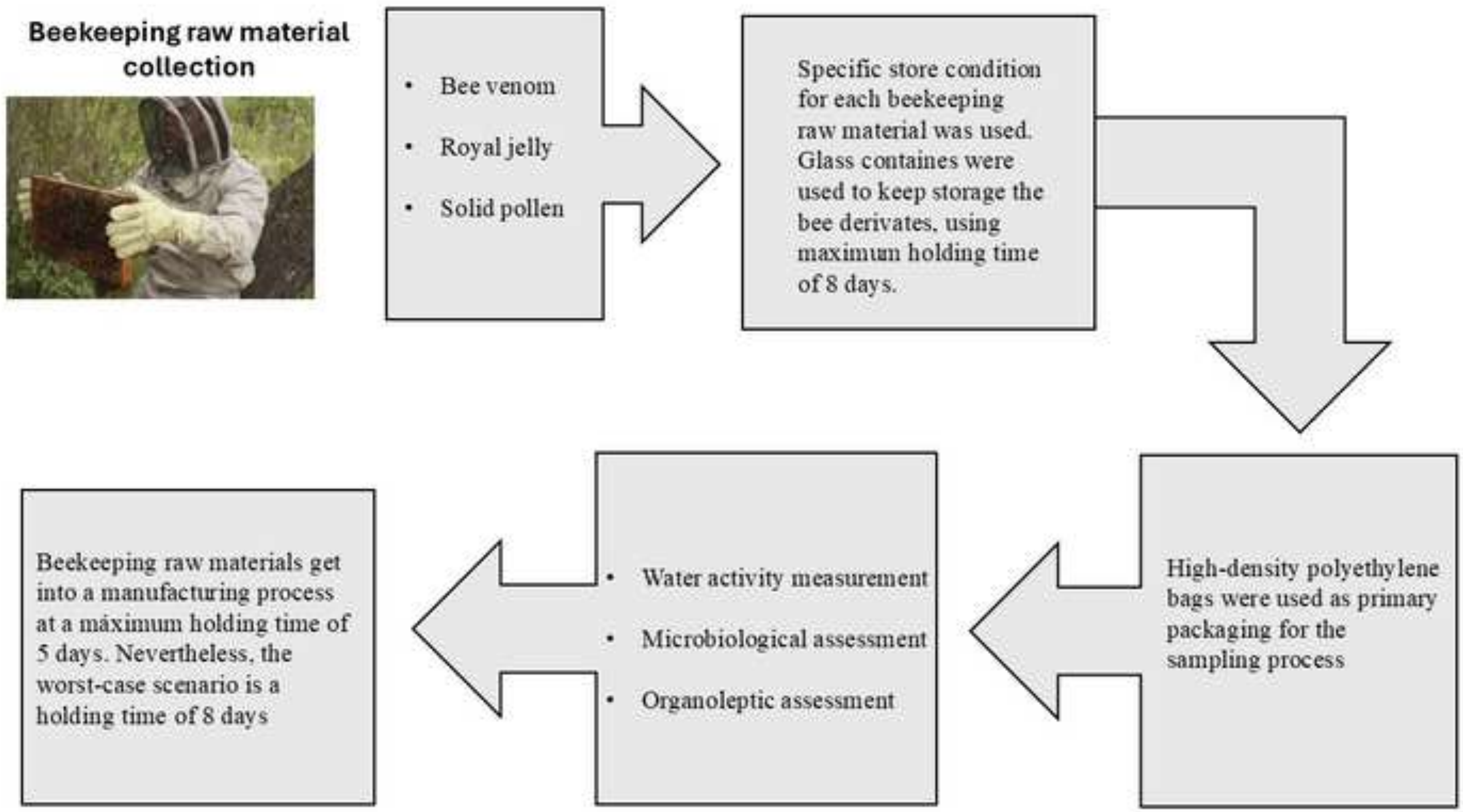
Beekeeping raw material collection
• Bee venom
• Royal jelly
• Solid pollen
Specific store condition for each beekeeping raw material was used. Glass containes were used to keep storage the bee derivates, using maximum holding time of 8 days.
High-density polyethylene bags were used as primary packaging for the sampling process
• Water activity measurement
• Microbiological assessment
• Organoleptic assessment
Beekeeping raw materials get into a manufacturing process at a máximum holding time of 5 days. Nevertheless, the worst-case scenario is a holding time of 8 days