

Production and characterization of powder from drone brood of honeybees (*Apis mellifera*)

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ABSTRACT

Drone bee brood are a natural source of food with potential for human nutrition. This work aims to investigate the production and extraction of the drone bee brood (larvae and pupae), as well as the production of powder from this insect for food applications. To produce the drone brood (DB), in some hives of the colony appropriate empty frames were placed. Some measurements were done in the hive to monitor the global wellbeing of the colony, namely, temperature, relative humidity, and weight. After approximately one month, the frames were removed from the hives and frozen. The yield of DB by frame was 38%. For the extraction of the drone brood, different methods were tested, being the best ones the cold room extraction (~ 6.5 °C), on a cold surface extraction (~ 11 °C), and in boiling water extraction (100 °C). The results show that the extraction efficiencies were 74.3% (336.3 s), 62.5% (246.8 s) and 84.9% (332.0 s), for extraction in a cold room, on a cold surface, and in boiling water, respectively. After extraction, two drying processes were tested: evaporation and freeze-drying, being the last one the recommended, preserving in a better way the appearance and composition of the DB. DB powders presented different colour characteristics according to the extraction method used. The powder obtained by boiling extraction was darker than the one produced with cold extraction. Powders are rich in protein, fat and carbohydrates, being the fat content quite high for DB powders produced by boiling extraction method.

1. Introduction

The world population is continually increasing and is expected to exceed 9.7 billion by 2050 (UN, 2024). Consequently, the food supply and production also need to increase. Ensuring the human right to adequate food is a premise that has emerged in line with the need to preserve natural resources and the balance of ecosystems, especially in the context of the climate change expected in the short and long term. Until now, animal protein production has been based on models in which water consumption and greenhouse gas emissions are excessive and have a very negative impact, particularly because of the large expanse of land that is occupied. According to the Food and Agriculture Organization of the United Nations (FAO), 77% of the area used in agriculture is dedicated to animal production (WEF, 2019).

Currently, new sources of animal protein are being considered, namely insect production, as alternative sources of protein for animal and human food. The FAO recognizes the use of insects as a sustainable

alternative for animal production and recommends the use of insects as a nutritional source for animals and humans since 2014 (Lumanlan et al., 2022). Insects are already commonly consumed as food or included in food products in many regions of the world. Particularly, honeybee brood are already consumed in several parts of the world, most especially in tropical areas (Ghosh et al., 2021).

In Portugal, the insect production sector has been growing and some initiatives have emerged to produce insects for animal feed and human food, using majority the cricket or tenebrio. In this context, it is important to identify other possible sources of insect protein, such as the drone brood (DB) of *Apis mellifera*. These DB (larvae and pupae) can be used for food, and at the same time contribute with additional source of income from the hive (Haber et al., 2019; Krell, 1996).

Beekeeping is a fundamental activity for agriculture, not only for the indispensable role played by bees in pollination, but also as a source of income for farmers. In addition to honey, drone production can be a source of additional income. The production of DB and their removal

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from the hive can have advantages for welfare of the hive. Current evidence indicates that the practice of DB removal is a sustainable and effective measure to control *Varroa* spp. mite (Calderone, 2005). This is relevant, since, *Varroa* spp. mite is a parasite that causes major losses in beekeeping worldwide, causing colony losses and reducing bees' productivity (Aldea & Bozinovic, 2020). However, the introduction of DB frames to reduce *Varroa* spp. infestations and produce DB needs proper training of beekeepers. The use of DB removal instead of chemical substances reduces pesticide costs and increases revenue for the beekeepers, who gain a new beehive product (Al-Ghamdi et al., 2021; Guiné et al., 2022).

Insect farming, marketing, and commercialization are experiencing considerable growth (Guiné et al., 2021), however there are several challenges related to using honeybee brood. Thus, the production of DB, and the techniques used for their removal from the hives and their extraction from the frames are factors that can directly impact their quality as well as the yield of extraction. Hence, it is important to the use of DB as a valued added product (or by-product) of the beekeeping sector (Guiné et al., 2022).

Honeybee DB is rich in macronutrients (high content of protein, fat and carbohydrates) (Bogdanov, 2016) and they can offer an alternative source of nutrients for human consumption (Finke, 2005; Guiné et al., 2022). According to Huis (2013) the high nutritional value of honeybee larvae and pupae can be compared to beef according to their protein quality and quantity. Moreover, DB is a bee product used and valued in the treatment of many diseases, including male infertility and women's menopausal disorders (Sidor, Mišek, Tomczyk et al., 2021).

One way to use the drone larvae is the production of powder with high protein content, rich in fibre and micronutrients such as iron, magnesium, manganese, phosphorus, and zinc, which can be used in the development of new products, for example, by incorporating it in the manufacture of products based on cereals, milk or others (Dossa et al., 2023; Ghosh et al., 2020). As an example, a recent study was published on the use of DB powder as a viable substitute for milk powder in fermented dairy products, particularly yoghurts (Neves et al., 2024). Furthermore, other DB product is also produced, the drone brood homogenate, known as apilarnil obtained by the collection of DB, which may be define as the male equivalent of royal jelly (Özkök & Erdem, 2017). It is used as a dietary supplement, and is considered a very active therapeutic agent (Sidor & Džugan, 2020), recommended mainly for the problem of male infertility (due to its high hormonal activity), and therefore recommended as dietary supplement with limited daily intake (Džugan et al., 2023).

In spite of the benefits and possible applications of DB, it has not yet been included on the list of edible insect species as a novel food (European Food, Commition, wd), thus it is not possible mention the DB powder as food or food ingredient at this moment, requiring authorisation from the EFSA (European Food Safety Authority), and its own regulations, as with other edible insects.

The main objectives of the present work were the follow and optimize the DB production; compare different extraction methods of DB; test different drying processes of DB; and produce DB powder that could be applied as a food product for human consumption.

2. Materials and methods

2.1. Drone brood production and collection

DB were produced in the apiary of Agrarian School of the Polytechnic University of Viseu. The apiary is located on a large property, with forest cover, grazing fields, a pond, and horticultural production, resulting in a multi-flower honey. To produce DB one frame of drone combs was placed inside each hive to provide the queen ample space to lay drone brood. The temperature and relative humidity of the hives were then measured and monitored, starting on 24 May 2023, and during, one month. The production started at the beginning of the spring season, as

the availability of food from nature reaches its maximum. The complete frames (combs with DB and wax) were removed after three weeks. The inclusion of empty frames in the hive induces the worker bees to build new drone combs and encourages the queen to lay new eggs to produce drone brood (Boes, 2010; Gross, 2018). In each hive, drone frames were placed, one frame per hive.

Once hormonal activity of drone brood changes during its development, knowing the age of the brood collection is crucial for consumer safety (Sidor, Mišek, Zagała et al., 2021). Thus, the collection of DB was made before the pupae's eyes become pink (Fig. 1), between the 5 and 9 days after the queen lay the eggs, since after that the chitin amounts will be increased, compromising their organoleptic quality and possible gastronomic utilization (Guiné et al., 2022; Jensen et al., 2019).

In the process of producing and collecting DB, certain procedures and precautions must be followed in terms of hygiene and good practice. Thus, the brood combs were harvested, without treatment with toxic substances, and introduced into clean containers for transportation to avoid contamination or physical damages. Moreover, after harvest the frames were immediately transported to main installations, catalogued, placed, and stored in a freezer. DB is a very unstable material and oxidizes quickly (observed as darkening), which is why it is very important that combs must be frozen as soon as possible after being removed from the hive (Sidor, Mišek, Tomczyk et al., 2021).

Hygiene conditions were considered, with the frames being wrapped in plastic film to ensure the integrity of the combs and avoid contamination.

The general conditions of five hives were evaluated using temperature and humidity data loggers' sensors (Model 174-H from Testo, Titisee-Neustadt, Germany). Thus, for each hive, one sensor was placed inside the hive to monitor the temperature and humidity conditions during the first days of DB production. A cotton cloth was used to protect the sensors inside the hive. One other sensor was located outside, monitoring the environment conditions.

The yield of DB production from each frame (comb with DB) harvested from the hives was determined by Eq. (1):

$$\text{Yield by frame}_{DB}(\%) = \frac{m_{DB}}{m_{\text{comb with DB}}} \times 100 \quad (1)$$

The frames harvest in the apiary were then transported to the facilities. Some of the frames were immediately used in the extraction tests and others were frozen at -20°C .

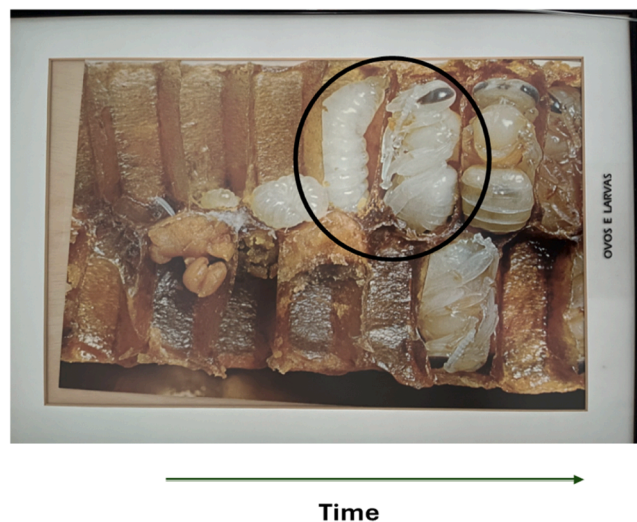


Fig. 1. Development cycle of DB.

2.2. Drone brood extraction

The drone brood combs consist of drone brood and bee wax. The brood needs to be separated from the wax, since the ingestion of wax in large quantities is not recommended (EFSA, 2007). Several methods were applied to extract the DB, considering that they are fragile, and must be kept as intact as possible. Based on the methods proposed by Jensen et al. (2019), four different methods were tested for the removal of DB from the comb cells of the harvested frames, based on the simplicity and the possibility of replication by beekeepers:

- Open the comb cells and shake the honeycomb, for DB to come out and fall on a clean surface. This process was applied to combs placed at room temperature (immediately after being collected from the hive) and to froze combs.
- Immerse the open combs in a container with water (at room temperature) and shake to separate the DB. This process was also applied to combs placed at room temperature (immediately after being collected from the hive) and to froze combs.
- Extraction in boiling water, using combs maintained at room temperature (immediately after harvest, unfrozen) (C1) and frozen (C2). For the hot extraction in boiling water, combs containing DB were broken into smaller pieces. These pieces were weighed and placed in a goblet with boiling water, for 5 min. With the aid of a strainer, the drone larvae and pupae were removed, separating them as much as possible from the wax and other materials (which melted), and weighed again. The extracted DB were then frozen until further use.
- Extraction at freezing temperature, in two conditions: extraction above a cold surface, at ambient temperature (~ 21 °C) (D1), and inside a cold room (~ 6.5 °C) (D2). For this extraction just frozen combs were used. The DB extractions were done manually.

In the last method (D - extraction at freezing temperature), in a first stage, the extraction was carried out in a room at temperature close to 21 °C, but it was done over a cold surface (tray) maintained in contact with a cooling bath of a common beer draught equipment with some adaptations. This type of equipment was chosen because it is relatively easy to acquire, easy to adapt and easy to use. All these aspects could be important for the future use of this prototype by beekeepers. Moreover, in this equipment, the evaporator coil is immersed in a water container that cools down to a temperature of approximately 0 °C. However, it was noticed that due to the high ambient temperature, the DBs thawed quickly, which was not what was intended. To solve this problem, a cupola (an acrylic bell) was designed to be placed on the cold surface of the refreshing equipment (Fig. 2), creating a barrier between the cold surface and the environment, which prevented the larvae from thawing during extraction. Moreover, this cupola allows the handling of the combs for the extraction and separation of the drones, because it has an access window with two openings where the operator can put their hands in and handle the frozen combs.

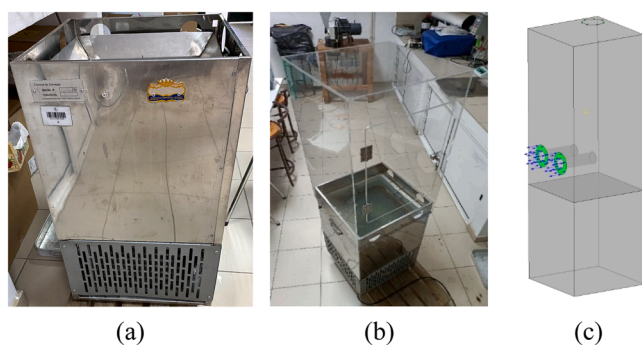


Fig. 2. Images of freezing equipment: (a) with the envelop cupola, (b) without the cupola, and (c) schematic representation of the equipment.

To find out about the temperature variations of the air inside of the acrylic box, the temperature of the air inside, the temperature of the cooling water inside, and the temperature of the stainless-steel surface were all monitored. Only with the cooling system working, the temperature of the bath water was measured, and it was verified that a constant temperature close to the freezing temperature (around 0 °C) was ensured. The surface temperature of the tray was also measured, and it was verified that it remained at approximately 1 °C. Under those conditions, the air temperature inside the acrylic box was close to 11 °C. The monitoring of the air temperature inside and outside the acrylic box was done with a data logger (Model 174-H from Testo, Titisee-Neustadt, Germany), whose results are shown in Fig. 3. The lower temperature of the surrounding air improves the integrity of the comb and the drones, and consequently the final product.

When extracting the DB at frozen temperatures, it was noticed that the brood and wax defrost quickly, and so it is advised to limit the amount of material that can be handled at one time to small pieces, and when that happens it makes it difficult to separate the brood and the wax (Al-Ghamdi et al., 2021; Jensen et al., 2019). Thus, the comb was broken into small pieces of approximately 90 g each. These small comb pieces were weighed ($m_{initial}$), and after the drone larvae were extracted from the comb (completely clean without wax residues), they were also weighed.

Both the drone larvae (m_{larvae}) and the residues were then weighed to determine the extraction efficiency (Equation (2)).

$$\text{Extraction efficiency (\%)} = \frac{m_{larvae}}{m_{initial}} \times 100 \quad (2)$$

After the extraction of DB, they were immediately stored in frozen conditions since this can impact the product's quality and safety for human consumption.

The extraction methods were evaluated by extraction efficiency, extraction time and yield. Seven experiments were done for each extraction method.

2.3. Powder production

After the extraction of the DB, which were previously frozen, the production of the powder contemplates several processing stages: drying, grinding, and sieving.

2.3.1. Drying processes

Two drying processes were tested to dehydrate the DB: hot air drying and freeze-drying (called lyophilization).

The hot air-drying process was done at 40 °C for 48 h in a ventilated chamber (Binder GmbH, Tuttlingen, Germany – model FD 115), with an air flow of 300 m³/h.

For the freeze-drying of DB, a freeze-dryer Christ - Alpha 1-4 LSC (Christ, Osterode am Harz, Germany) was used, with a condenser temperature of - 48.8 °C, average pressure of 1.54 mbar, and an ice condenser temperature of -55 °C.

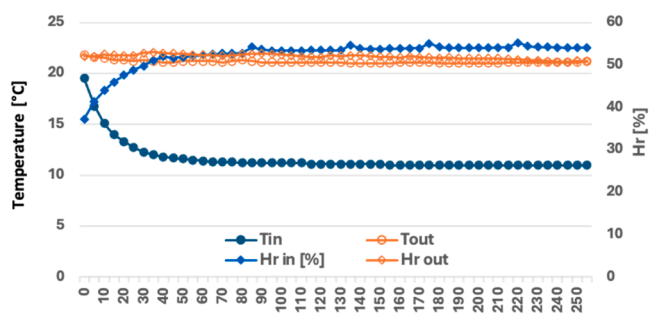


Fig. 3. Temperature (T) and Relative humidity (Hr) evolution, inside (in) and outside (out) of the air of the acrylic box.

The time required for an effective freeze-drying, and the final equilibrium moisture content of freeze-dried DB were determined. Samples weighing 30 g were placed in the freeze-dryer for increasing time intervals (multiples of one hour), then removed from the equipment for analysis.

For measuring the moisture content, the samples were placed in a convective drier to dry until constant weight. The water activity (a_w) was also measured with a hygrometer (Hygroskop model BT-RS1, from Rotronic, Bassersdorf, Switzerland), coupled to an isothermal bath. This procedure was repeated for three DB extraction methods, and all measurements (moisture and a_w) were done in triplicate for each moment along the drying process.

The powder production efficiency per frame was also investigated. For this purpose, frozen drone samples (approximately 250 g) were freeze-dried, grounded, and sieved separately. At each processed stage they were also weighed, and the yields of each operation were calculated. The yield of freeze-drying operation was determined according to Eq. (3):

$$\text{Freeze - drying yield}(\%) = \frac{m_{\text{after freeze-drying}}}{m_{\text{before freeze-drying}}} \times 100 \quad (3)$$

2.3.2. Grinding and sieving

After freeze-drying, the DB were subjected to grinding operation in a grinder (La Moulinette 1, 2, 3 AD560120, Moulinex, Windsor - Datchet, United Kingdom) and then sieved through a 0.5 mm mesh stainless steel sieve (Retsch GmbH, Haan, Germany).

The yields of grinding and sieving were determined according to Eqs. (4) and (5):

$$\text{Grinding yield}(\%) = \frac{m_{\text{after grinding}}}{m_{\text{before grinding}}} \times 100 \quad (4)$$

$$\text{Sieving yield}(\%) = \frac{m_{\text{after Sieving}}}{m_{\text{before Sieving}}} \times 100 \quad (5)$$

2.3.3. Mathematical modelling of the drying kinetics

It is common usage to describe the drying of agricultural and food products by thin layer equations, which describe the process in a combined way, regardless of the mechanisms involved, such as mass and heat transfer. These equations express variations in moisture content along drying as functions of some parameters like the drying constant or the lag factor, being these responsible for complex combined effects of several transport phenomena occurring during drying (Guiné, 2018).

A great diversity of thin layer equations can be found in the scientific literature to successfully explain the drying of various products of the agrifood sector (Barroca & Guiné, 2021; Ferrão et al., 2019). Most of the thin layer models include a dimensionless variable, the moisture ratio (MR), defined as follows:

$$MR = \frac{W_t - W_e}{W_0 - W_e} \quad (6)$$

where W_t is the moisture content at any time t , W_e is the equilibrium moisture content, and the W_0 is the initial moisture content, all expressed on a dry basis.

Table 1 presents some selected models among the most used to

Table 1
Selected mathematical models used to fit the drying kinetics (Barroca & Guiné, 2021; Ferrão et al., 2019).

Model name	Equation
Page	$MR = \exp(-k t^n)$
Modified Page	$MR = \exp[-(k t)^n]$
Henderson & Pabis	$MR = a \exp(-k t)$
Logarithmic	$MR = a \exp(-k t) + c$
Diffusional approach	$MR = a \exp(-k t) + (1-a) \exp(-k b t)$

describe the drying kinetics.

2.3.4. Mathematical modelling of the sorption isotherms

The GAB model is undoubtedly one of the most used and taken as reference to describe the desorption isotherms of many agricultural and food products. The GAB model (Table 3) includes three parameters with physical meaning (a_w is the water activity, W_e is the equilibrium moisture content, W_m is the water content of the monolayer) plus two temperature dependent functions (C and K), related to the energies of interaction between the first and farthest adsorbed molecules in individual sites of adsorption. Besides the GAB model, other equations found in the literature (Table 2) were also used to fit the experimental data of the desorption isotherms (Barroca & Guiné, 2021).

2.3.5. Statistical evaluation of the mathematical models

The fittings were made using the software SigmaPlot V 14.0, and evaluation of the suitability of the models was achieved by calculating some statistical indicators besides the regression coefficient (R), which were: the Mean absolute error (MAE), Root mean square error (RMSE), Standard error (SE), Sum of square errors (SSE) and Relative percent deviation (RPD), as defined by the following equations (Barroca & Guiné, 2021):

$$MAE = \frac{1}{N} \sum_{i=1}^N |V_{\text{exp},i} - V_{\text{pred},i}| \quad (7)$$

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^N (V_{\text{exp},i} - V_{\text{pred},i})^2} \quad (8)$$

$$SE = \frac{\sqrt{\sum_{i=1}^N (V_{\text{exp},i} - V_{\text{pred},i})^2}}{N - 1} \quad (9)$$

$$SSE = \frac{1}{N} \sum_{i=1}^N (V_{\text{exp},i} - V_{\text{pred},i})^2 \quad (10)$$

$$RPD = \frac{100}{N} \sum_{i=1}^N \frac{|V_{\text{exp},i} - V_{\text{pred},i}|}{V_{\text{exp},i}} \quad (11)$$

In the above equations N stands for the number of observations, and $V_{\text{exp},i}$ and $V_{\text{pred},i}$ represent the experimental and predicted values for the dependent variable at any observation i .

The RMSE compares differences between $V_{\text{exp},i}$ and $V_{\text{pred},i}$ and when these differences approach zero it is indicative that the prediction is close to the experimental data. RMSE provides information on the short-term performance of the correlations by allowing a term-by-term comparison of the actual deviation between the calculated and measured values. The RPD compares the absolute differences between $V_{\text{exp},i}$ and $V_{\text{pred},i}$, and should be as low as possible (Barroca & Guiné, 2021; Ferrão et al., 2019).

Table 2
Selected mathematical models used to describe the desorption isotherms (Barroca & Guiné, 2021).

Non explicit temperature dependent		
Model	Equation	Parameters
GAB	$W_e = \frac{w_m C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)}$	W_m, C, K
Oswin	$w_e = C \left(\frac{a_w}{1 - a_w} \right)^b$	C, b
Smith	$W_e = A + B \ln(1 - a_w)$	A, B
Chen	$aw = \exp(-A \exp(-B W_e))$	A, B
Explicit temperature dependent		
Model	Equation	Parameters
Chung & Pfof	$aw = \exp\left(-\frac{A}{R T} \exp(-B W_e)\right)$	A, B
Halsey	$aw = \exp\left(-\frac{A}{R T} \frac{1}{(W_e/W_m)^b}\right)$	A, W_m, b
Henderson	$aw = 1 - \exp(-C T W_e^b)$	C, b

2.4. Determination of physicochemical properties of DB powder

Chemical composition, including moisture, fat, protein, ash content and fibre of each DB powder were determined according to the AOAC (2020) methods (952.08, 948.15, 991.20, 923.03, and 985.29, respectively). Total sugars were determined by difference of fat, protein, and ash. All determinations were carried out in triplicate. Chemical analyses were performed using reagents with high analytical grade. Ultrapure water obtained from a Milli-Q purifier (Millipore, Eschborn, Germany), was used for the preparation of all solutions.

Water activity (a_w) was measured at 25 °C using a HS2 hygrometer (Rotronic, Switzerland).

The colour of the DB powders samples was assessed using a handheld tristimulus colorimeter (Chroma Meter-CR400, Konica Minolta, Chiyoda, Tokyo, Japan). The colour coordinates evaluated were: L^* that represent the lightness and varies from 0 (black) to 100 (white), a^* which takes negative values for green and positive for red, and b^* which is negative for blue and positive for yellow (Kim & Lee, 2009). A white tile ($L^* = 97.46$, $a^* = -0.02$, $b^* = 1.72$) was used as reference for calibration. Two samples from each type of extraction were analysed, and 35 measurements were taken in each sample to determinate the average of the coordinates L^* , a^* , and b^* .

3. Results and discussion

3.1. Drone brood production and hive monitoring

The results of the evolution of temperature and humidity inside and outside the hive are shown in Fig. 4. The initial moment corresponds to 00:00 h of the 1st day (24 May 2023), and the time is measured as time elapsed from the beginning of the monitoring. A large variation on outdoor air temperature comparing the night (lowest outside temperature) and day (highest outside temperature) periods. During the measurement, outdoor temperature varying from 7.5 °C and 38.7 °C, and relative humidity ranged from 24.2% to 62.9%, during the monitoring interval. The average values of outside temperature and relative humidity were 22.8 °C and 39.6%, respectively.

In the same monitoring period (Fig. 4), the indoor temperature and relative humidity values were always higher and with smaller variations between day and night periods. The lowest inside temperature was 23.9 °C and the maximum temperature inside the hive was 34.7 °C. The average values of inside temperature and relative humidity were 29.4 °C and 77.9%, respectively. Similar results were obtained for the other hives.

Despite the ambient temperatures, honeybees tend to maintain their own microclimate within beehives. To have a healthy bee brood and good honeybee workers it must be ensured that inside the hive the temperature is maintained in a range of 32–36 °C (Kovac et al., 2009).

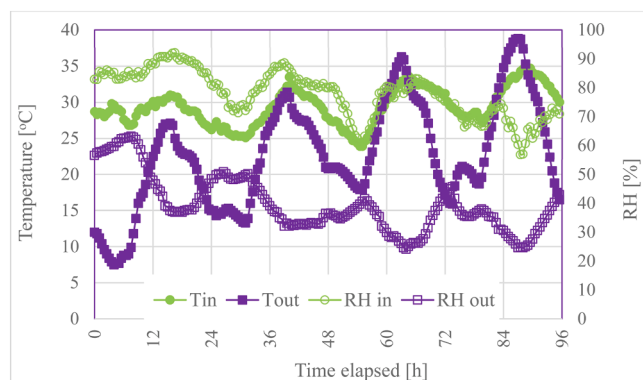


Fig. 4. Temperature (T) and Relative Humidity (HR) evolution inside (in) and outside (out) of the hive, between 24th and 28th of May 2023.

Furthermore, the temperature plays an important role in the length of the larval stage because brood development is dependent of temperature (Abou-Shaara et al., 2017). According to Li et al. (2016) temperatures of worker brood were higher than drone brood.

Relative humidity (RH) is another important parameter for DB development, particularly important for egg hatching. It is also controlled mainly by worker honey bees (Li et al., 2016). The eggs require a RH above 55% to hatch successfully, with the optimum RH range for normal hatching being from 90 to 95% (Abou-Shaara et al., 2017; Ziegler et al., 2022).

The present results confirm the good indoor thermal environment inside the hive, ensuring the well-being of the hive, and enhancing good development of the brood, including DB (Kovac et al., 2009; Szentgyörgyi et al., 2018).

Several authors mention that a good drone brood production is indicative of a good health of the colony. Drone production is also dependent on a good pollen supply to meet their protein demand (Jensen et al., 2019).

The yield in DB was determined by weighing the frames produced in the 5 hives (Table 3). The results show that there is a wide variation in terms of DB comb production, which is then reflected in the DB yield per frame. It was found that 1 Kg of frame corresponds to 788 g of comb with DB, and 297.5 g of DB, i.e. approximately 300 g. The yields ranged from 28.1% to 54.0%, and the average production per DB frame was 38%, in this case per hive, as each hive had one frame for DB production.

3.2. Extraction of drone brood

The extraction methods where the comb cells were open and shaken (Method A) were found not effective, either for the combs placed at room temperature or for the frozen combs. It was observed that only about 20 per cent of DB fell out of the comb, the rest stuck to the wax. Similar results were achieved with the extraction method where the combs were immersed in a container with water and shaken to separate the DB (Method B), both for combs placed at room temperature (immediately after being collected from the hive) and frozen combs. Considering the obtained results, these methods were not adopted for future tests.

Regarding the extraction of DB in boiling water (Method C), using unfrozen combs (C1) the process proved to be efficient (Table 4). However, this method was turned out because DB began to degrade quickly if they weren't extracted straight away, since the combs were not frozen. Thus, the quality of the DB extracted with this process was not guaranteed, despite its high yield, and the time for extraction was very similar to the extraction of frozen combs. As so, the extraction of DB in boiling water with unfrozen combs was abandoned. Alternatively, the extraction of DB in boiling water was made with frozen combs (Method C2), and this was the option considered for further investigation. Furthermore, several authors mention that the freezing of the combs seems to be the best way for conservation and extraction of DB (Jensen et al., 2019).

Extraction at freezing temperature (Method D) was carried out using frozen combs under two conditions: D1) extraction above a cold surface, at ambient temperature (~11 °C), and D2) inside a cold room (~6.5 °C).

Table 3
Yield of DB production from the frames.

Sample	Frame (g)	Comb with DB (g)	DB (g)	Yield by frame (%)
1	1106.96	869.06	376.43	43.3
2	1063.04	811.36	291.82	36.0
3	1269.36	993.53	531.03	54.0
4	1127.28	868.57	258.07	29.7
5	1507.38	1242.77	349.70	28.11
Mean	1214.8 ±	957.1 ± 173.0	361.4 ±	38.2 ± 10.7
±Sd	180.9		105.7	

Table 4
Extraction efficiency and time for boiling and freezing temperatures.

Extraction Method	Extraction efficiency ¹ (%) ²	Extraction time ¹ (sec)
C1 - Boiling (with unfrozen combs)	98.4 ± 1.1	290±80
C2 - Boiling (with frozen combs)	84.9 ± 4.0	300±74
D1 - Freezing - Cold surface	62.5 ± 4.5	207±33
D2 - Freezing - Cold room	74.3 ± 5.9	258±48

¹ Values are mean±standard deviation.

² Percentage as g DB/100 g comb.

To keep the drone larvae intact and prevent or delay their rapid thawing, in preliminary experiments, the extraction of the drones was done inside a refrigerated room, located in an industrial food facility. In these experimental trials, it was found that this environment was very uncomfortable for the person that manually extracted the DB, as the refrigerated chamber was kept at low temperatures, as mentioned before. This was identified as one of the major disadvantages of this procedure. The extraction efficiency and the time required to extract DB are presented in Table 4. This table also presents the results for the extraction of DB at the cold surface (method D1).

The extraction efficiency was higher in the boiling water (method C) than in both cases of cold extraction (method D). In the hot water extraction, although the drone larvae/pupae were carefully removed with a strainer, they brought with them some wax and other residues (Fig. 5). Although most of the wax had melted, there were still remnants of material (wax and other, unidentified materials) clinging to the larvae and pupae. This could be the reason for the higher extraction efficiency. Another reason for the higher efficiency is that in these cases the extracted drone larvae also bring some moisture, due to being immersed in the water.

The drone larvae extraction method at cold temperature (method D) presented a lower efficiency than extraction in boiling water method (C). The extraction over a cold surface method (D1) presented the lower extraction efficiency, but also corresponded to lower time needed for the extraction. The extraction efficiency in a cold room (D2) was higher than on cold a surface, but also caused greater thermal discomfort to the operator performing the extraction, as mentioned before. The DB extracted from both previous methods (method D cold surface/cold room), presented similar results considering the appearance of the material. The DB showed a clean appearance, maintaining its original shape (Fig. 5). Applying these manual extraction techniques, it was possible to remove all residues from the sample including the wax, resulting in a final product of good quality and appearance. Moreover, it seems that

the method of extracting DB at low temperature on a cold surface (method D1) could be the most convenient, because despite having a lower yield, it was found to maintain the good characteristics of the DB and the safety while also guaranteeing the well-being of the operator.

3.3. Drying of drone brood

After extracting the DB, they were subjected to two dehydration processes: evaporation under hot air at 40 °C and freeze-drying. The hot air-drying process was not effective, as the drone larvae and pupae did not dry out, but became sticky and soft. Even though they remained in the drying oven for several days, it was not possible to dry them. In addition, there was a marked darkening of the larvae, with some turning a very dark brown colour. On the other hand, freeze-drying proved to be an efficient process for drying the DB. In this way, the time required for an effective freeze-drying operation was determined, in this case until the moisture content and water activity stabilized (Fig. 6).

Fig. 6 shows that the different methods have a similar profile for water loss. The evolution of DB moisture content decreased along the time required for lyophilization. The D2 method had similar values in water activity and moisture content as D1 method, and lower than the C2 method considering water activity measures after 25 h. The moisture content stabilized after 18 h, reaching values between 5.4% and 8.4% of moisture, for cold room extraction (D2) and boiling extraction (C2), respectively. Regarding water activity, again the curves showed similar trends, and water availability gradually decreased. The most marked differences appeared after 18 h from the start of freeze-drying. In this case, boiling extraction (C2) showed the highest value of water activity ($a_w = 0.39$) and cold room extraction (D2) the lowest ($a_w = 0.24$). Knowing that the standard values for water activity in terms of preservation and food safety should be lower than 0.6, it can be observed from the results obtained that after 15 h of freeze-drying this value was reached for all the extraction methods. Thus, for freeze-drying to be effective, the samples must remain in the freeze-dryer for at least 15 h

3.3.1. Modelling of the drying kinetics

For the modelling of the drying kinetics the data were expressed in terms of moisture ratio, as defined by Eq. (4), based on values of dry basis moisture content. Fig. 7 presents the variations of the dry basis moisture content, W, and moisture ratio, MR, for the freeze-drying of DB extracted by different methods. The initial values of W were 2.98, 2.82 and 2.68 kg water/kg dry solids, respectively for methods C2, D1 and D2. The drying curves obtained for the freeze-drying process of DB for the three extraction methods show that the values of W and MR decreased linearly with time for the initial drying process (until ~10,000 s, 3 h). For higher drying times, however, the decrease in W and MR was slower, which is a characteristic of the falling rate period. It was further observed that the drying ended around 65,000 s (~18 h), achieving the equilibrium moisture content, with values of W and MR practically zero.

To fit the experimental drying data some models commonly used for convective drying were tested for the present data on freeze-drying of DB. These are semi-empirical models, which are based on the dimensionless variable moisture ratio (MR) as expressed in the formulas presented in Table 1. The goodness of the fit for was evaluated in all cases according to the statistical indicators defined by Eqs. (5) to (9). The parameters and estimated and statistics for the fitting of kinetic data for all cases (different models and different extraction methods) are presented in Table 5. Considering the statistics presented in Table 5, the values of R varied from a minimum of 0.9686 to a maximum of 0.9982, indicating fittings with very good regression coefficients, very close to one. On the other hand, the values of the other statistical indicators were very low, tending to zero: MAE varying in the range 0.0128 – 0.0783, RMSE in the range 0.0190 – 0.1055, SE in the range 0.0489 – 0.0255, and SSE in the range 0.0003 – 0.0112. The values of RPD were between 16.5452 and 93.8542. However, the best models, with highest values of

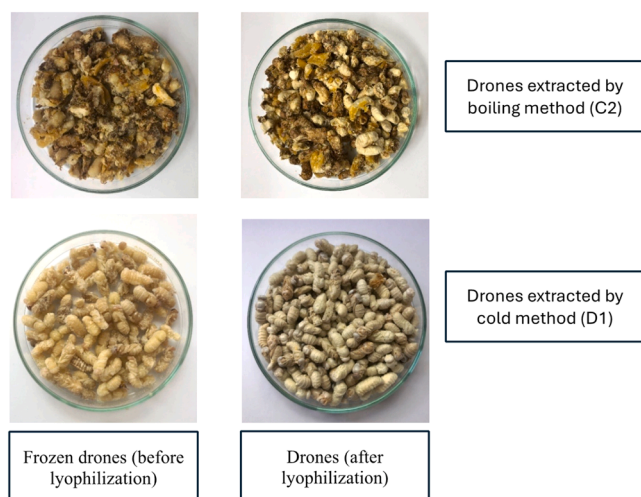


Fig. 5. Appearance of drone brood before and after lyophilization, extracted from cold (D1) and hot methods (C2).

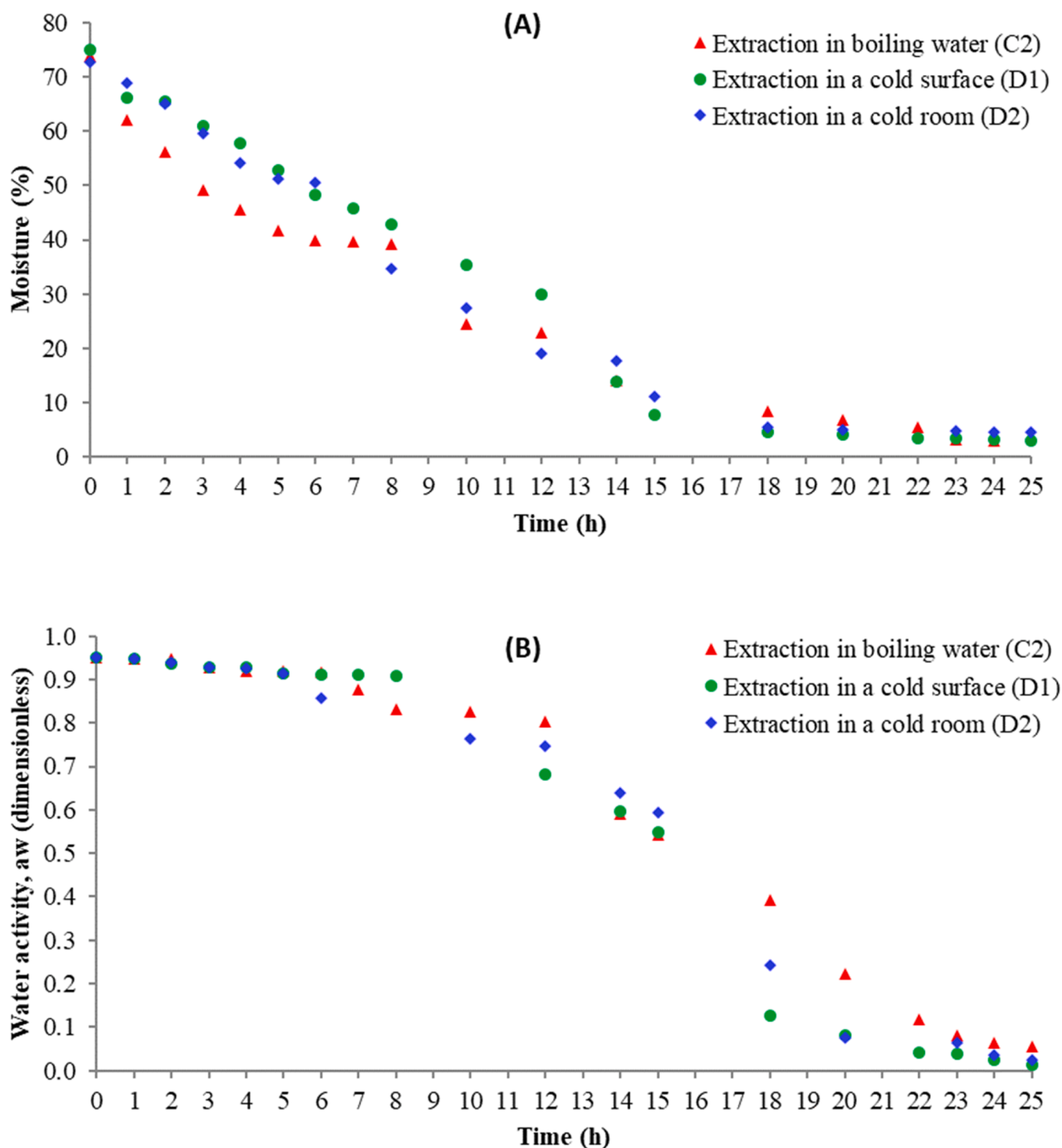


Fig. 6. Evolution of moisture content (A) and water activity (B) along the freeze-drying processes of DB extracted by different extraction methods.

R and lowest values of RPD, for the freeze-drying of DB with different extraction methods were the following, with the respective equations, where time (t) is in seconds:

- For the drying with extraction method C2 – kinetic model logarithmic ($R = 0.9911$, $RPD = 27.8701$):

$$MR = 0.9199 * \exp(-4.8520e - 5 * t) - 0.0069 \tag{12}$$

- For the drying with extraction method D1 – kinetic model diffusional approach ($R = 0.9972$, $RPD = 18.8023$):

$$MR = 0.4605 * \exp(-0.0004 * t) + (1 - -0.4605) * \exp(-0.0004 * 0.0996 * t) \tag{13}$$

- For the drying with extraction method D2 – kinetic model logarithmic ($R = 0.9982$, $RPD = 16.5452$):

$$MR = 0.9954 * \exp(-5.3000e - 5 * t) + 0.0018 \tag{14}$$

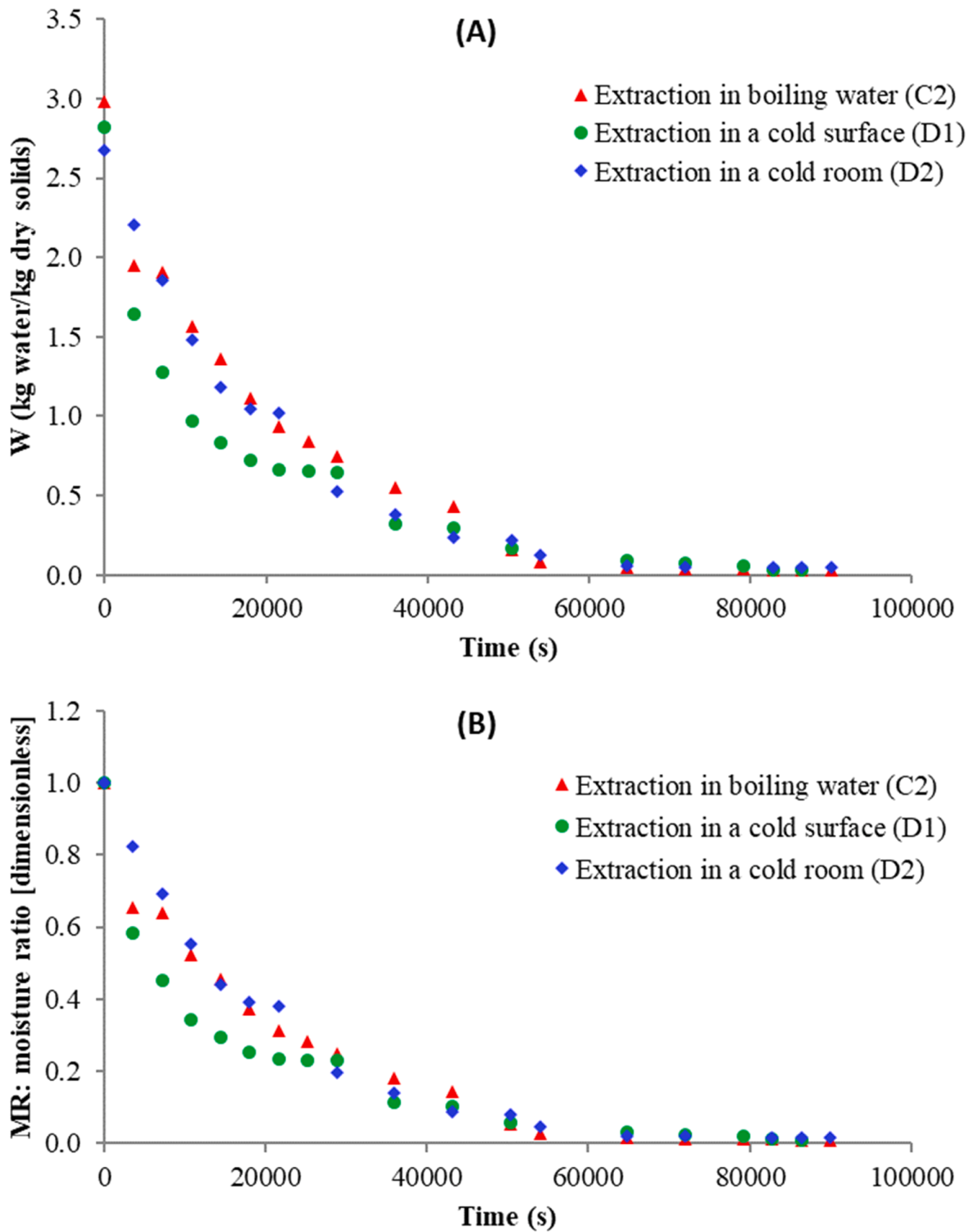


Fig. 7. Variations of the dry basis moisture content (A) and moisture ratio (B) along freeze-drying of DB extracted by different methods.

Table 5

Parameter estimation and statistics for fitting the kinetic data with the five models considered, for the freeze-drying of DB obtained with different extraction methods.

Model / Extraction method	Parameters			Statistics ¹					
	k	n		R	MAE	RMSE	SE	SSE	RPD
Page									
Boiling water (C2)	0.0003	0.8238		0.9920	2.4528e-2	3.8362e-2	9.2899e-3	1.4717e-3	93.8542
Cold surface (D1)	0.0041	0.5937		0.9952	1.7733e-2	2.4137e-2	6.2198e-3	5.8257e-4	43.0588
Cold room (D2)	5.6604e-5	0.9932		0.9981	1.2977e-2	1.9098e-2	4.9214e-3	3.6473e-4	17.3493
Modified Page									
Boiling water (C2)	5.7053e-5	0.8238		0.9920	2.6381e-2	3.4867e-2	8.4436e-3	1.2157e-3	72.6124
Cold surface (D1)	9.3704e-5	0.5937		0.9952	1.8223e-2	2.3942e-2	6.1698e-3	5.7324e-4	45.3780
Cold room (D2)	5.2956e-5	0.9932		0.9981	1.2963e-2	1.9100e-2	4.9220e-3	3.6481e-4	17.3325
Henderson & Pabis									
Boiling water (C2)	4.9639e-5	0.9163		0.9910	2.3314e-2	3.6944e-2	8.9463e-3	1.3648e-3	32.7696
Cold surface (D1)	6.6904e-5	0.8632		0.9686	4.8683e-2	6.1746e-2	1.5911e-2	3.8125e-3	39.2330
Cold room (D2)	5.2712e-5	0.9964		0.9981	1.3082e-2	1.9057e-2	4.9110e-3	3.6319e-4	17.2728
Logarithmic									
Boiling water (C2)	k	a	c	R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	4.8520e-5	0.9199	-0.0069	0.9911	2.2732e-2	3.6816e-2	8.9155e-3	1.3554e-3	27.8701
Cold surface (D1)	8.4203e-5	0.8490	0.0513	0.9748	4.6217e-2	5.5297e-2	1.4250e-2	3.0577e-3	79.1688
Cold room (D2)	5.3000e-5	0.9954	0.0018	0.9982	1.2960e-2	1.9102e-2	4.9226e-3	3.6490e-4	16.5452
Diffusional approach									
Boiling water (C2)	k	a	b	R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	0.0042	0.1722	0.0105	0.9961	7.8281e-2	1.0546e-1	2.5538e-2	1.1122e-2	55.2011
Cold surface (D1)	0.0004	0.4605	0.0996	0.9972	1.3583e-2	1.9009e-2	4.8984e-3	3.6133e-4	18.8023
Cold room (D2)	5.3000e-5	1.0000	-2.8046	0.9982	1.2840e-2	1.9036e-2	4.9056e-3	3.6238e-4	17.0881

¹ Statistics: R = Regression coefficient, MAE = Mean absolute error, RMSE = Root mean square error, SE = Standard error, SSE = Sum of square errors, RPD = Relative percent deviation.

The values of the drying constant, parameter k in all kinetic equations, represent the combined effects of various transfer phenomena occurring during drying. From the Eqs. (12) to (14), the values of the drying constant were, respectively for C2, D1 and D2 the following 0.000048 s⁻¹, 0.000400 s⁻¹ and 0.000053 s⁻¹. According to these values, the highest drying constant was 0.0004 for the drying with extraction method D1 and fitting with kinetic model diffusional approach. This indicates a faster drying in the initial phase of drying until the stabilization period, as seen by the steepest slope of the curve in Fig. 8 (green points and line). This Fig. 8 shows the fitting for each of the three dryings (with extraction methods C2, D1 and D2) with the corresponding best mathematical models.

3.3.2. Modelling of the sorption isotherms

The sorption isotherms are useful to predict shelf life and to assess the background of other related operations to obtain a stable product. Fig. 9 presents the changes in the equilibrium moisture content as a function of water activity, corresponding to the desorption isotherms for

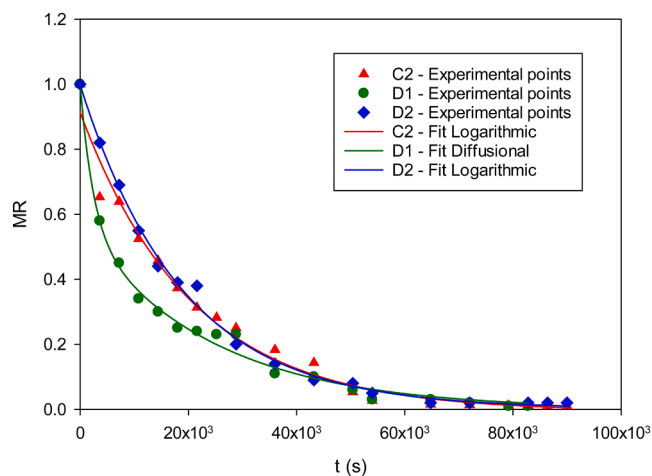


Fig. 8. Fitting of the kinetic data for the three drying experiments (extraction methods C2, D1 and D2) with mathematical models.

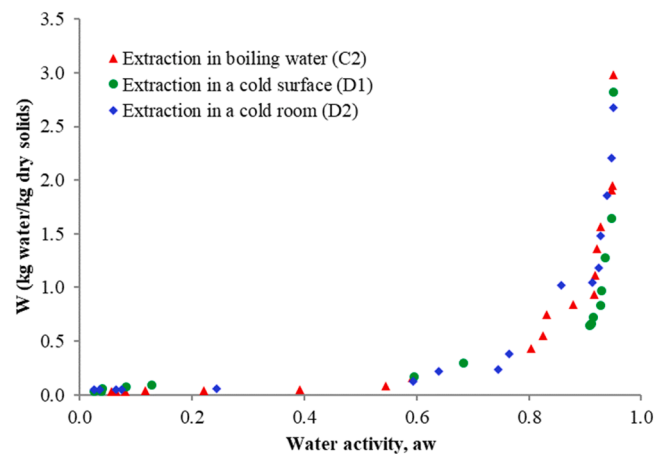


Fig. 9. Desorption isotherms for the freeze-drying of DB with different extraction methods.

the three freeze-drying essays with DB obtained with three extraction methods. According to the classification of five general types of sorption isotherms proposed by Brunauer et al. (1940), the sorption curves obtained are of type III isotherms, which is one of the most typical for food systems.

The three curves in Fig. 9 show that the equilibrium moisture content was practically zero for values of a_w below 0.6 and increased with water activity for values of a_w over 0.6. The curves show two different regions in the isotherms: a) at intermediate water activities moisture content increased more slowly with water activity, and this region is known as the multilayer sorption region; b) at high water activity levels, above 0.9, the water content increased sharply with water activity due to capillarity, being this known as the capillary condensation region (Barroca & Guiné, 2021; Ferrão et al., 2019). The slopes of the three curves for this high water activity level are very similar.

The experimental values of the equilibrium moisture content as a function of a_w, for the three extraction methods, were fitted by the sorption isotherm models presented in Table 2. The goodness of fit for each sorption model were again evaluated based on statistical indicators R, MAE, EMSE, SE, SSE and RPD. Table 6 presents the parameters of the models used to fit the experimental data as well as the corresponding

Table 6

Parameter estimation and statistics for fitting the sorption isotherms with the seven models considered, for the freeze-drying of DB obtained with different extraction methods.

Model / Extraction method	Parameters			Statistics ¹					
GAB	W_m	C	K	R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	0.0789	-7.5204e5	1.0178	0.9689	1.4391e-1	2.0419e-1	4.9446e-2	4.1692e-2	64.3014
Cold surface (D1)	0.0339	-1.3271e2	1.0382	0.9737	9.4933e-2	1.6579e-1	4.4210e-2	2.7486e-2	26.2530
Cold room (D2)	0.0771	3.2089e1	1.0204	0.9880	8.0001e-2	1.3040e-1	3.4773e-2	1.7003e-2	18.7311
Oswin	C	b		R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	0.0820	1.1340		0.9674	1.2851e-1	2.0879e-1	5.0560e-2	4.3592e-2	31.9351
Cold surface (D1)	0.0035	2.21111		0.9613	1.1913e-1	1.9915e-1	5.3106e-2	3.9660e-2	50.4644
Cold room (D2)	0.0785	1.1630		0.9841	1.0479e-1	1.5006e-1	4.0016e-2	2.2518e-2	38.1674
Smith	A	B		R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	-0.2459	-0.6704		0.8912	2.7914e-1	3.7409e-1	9.0590e-2	1.3994e-1	198.1015
Cold surface (D1)	-0.0953	-0.4811		0.7869	3.0602e-1	4.4585e-1	1.1889e-1	1.9878e-1	112.6509
Cold room (D2)	-0.2169	-0.6864		0.9095	3.0091e-1	3.5153e-1	9.3740e-2	1.2357e-1	173.2719
Chen	A	B		R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	1.8502	5.1785		0.9451	9.7229e-2	1.1272e-1	2.7296e-2	1.2706e-2	49.6297
Cold surface (D1)	4.0518	9.4947		0.9789	6.9576e-2	7.9279e-2	2.1141e-2	6.2852e-3	29.0545
Cold room (D2)	3.5331	10.3408		0.9645	8.3799e-2	9.6221e-2	2.5659e-2	9.2584e-3	57.4831
Chung & Pfost	A	B		R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	3.4303e3	5.1785		0.9451	9.9189e-2	1.1309e-1	2.7386e-2	1.2790e-2	53.3325
Cold surface (D1)	7.5121e3	9.4947		0.9789	7.1956e-2	7.9573e-2	2.1219e-2	6.3318e-3	34.2553
Cold room (D2)	6.5503e3	10.3408		0.9645	8.5866e-2	9.6550e-2	2.5747e-2	9.3219e-3	63.3627
Halsey	A	W_m	b	R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	1.2065e2	1.5001	0.8943	0.9852	4.3799e-2	5.9800e-2	1.4481e-2	3.5761e-3	28.0772
Cold surface (D1)	5.5872e1	1.4999	1.4242	0.9946	2.9722e-2	4.0785e-2	1.0876e-2	1.6634e-3	22.7771
Cold room (D2)	7.8777e1	1.4999	1.1687	0.9883	5.0428e-2	5.6080e-2	1.4955e-2	3.1450e-3	48.3804
Henderson	C	b		R	MAE	RMSE	SE	SSE	RPD
Boiling water (C2)	0.0121	0.7376		0.9675	7.0518e-2	8.7512e-2	2.1192e-2	7.6583e-3	44.4692
Cold surface (D1)	0.0208	1.2259		0.9814	6.1657e-2	7.4598e-2	1.9893e-2	5.5648e-3	45.3125
Cold room (D2)	0.0390	1.3710		0.9674	8.3713e-2	9.2511e-2	2.4670e-2	8.5583e-3	66.3461

¹ Statistics: R = Regression coefficient, MAE = Mean absolute error, RMSE = Root mean square error, SE = Standard error, SSE = Sum of square errors, RPD = Relative percent deviation.

statistical indicators for each model and extraction method used to obtain the DB. Based on the statistical parameters in Table 6, the values of R varied from 0.7869 to 0.9883, indicating fittings with acceptable or very good regression coefficients, although lower than for the kinetic study. However, the values of the other statistical indicators were very low, similarly to what was observed for the kinetic study, with some exceptions: MAE varying in the range 0.0297 – 0.3060, RMSE in the range 0.0408 – 0.4459, SE in the range 0.0109 – 0.1189, and SSE in the range 0.0017 – 0.1988. The values of RPD were between 18.7311 and 198.1015. The highest values of RPD and lowest values of R were obtained for model Smith, which proved to be less adequate to fit the experimental data of the sorption isotherms in the present case. In conclusion, the best models, with highest values of R and lowest values of RPD, to describe the sorption isotherms in freeze-drying of DB with different extraction methods were those in the Equations below:

- For the drying with extraction method C2 – Halsey model for isotherms (R = 0.9852, RPD = 28.0772):

$$aw = \exp\left(-\frac{120.65}{8.314 \times 233} \frac{1}{(W_e/1.5001)^{0.8943}}\right) \quad (15)$$

- For the drying with extraction method D1 – Halsey model for isotherms (R = 0.9946, RPD = 22.7771):

$$aw = \exp\left(-\frac{55.872}{8.314 \times 233} \frac{1}{(W_e/1.499)^{1.4242}}\right) \quad (16)$$

- For the drying with extraction method D2 – GAB model for isotherms (R = 0.9880, RPD = 18.7311):

$$w_e = \frac{0.771 \times 32.089 \times 1.0204a_w}{(1 - 1.0204a_w)(1 - 1.0204a_w + 32.089 \times 1.0204a_w)} \quad (17)$$

Fig. 10 shows the fitting for each of the three isotherms (with extraction methods C2, D1 and D2) and the fit with the corresponding best mathematical models.

3.4. Powder production from drone brood

The powder production efficiency was also investigated for the different steps separately: freeze-drying, grinding, and sieving. The results obtained were very similar for all the applied methods, and Table 7 shows the results for the extraction method of frozen DB on a cold surface (D1).

The results in Table 7 show that the yield of freeze-drying of DB was on average 28%. As expected, the grinding yield was close to 100%, since powder losses are small and only those resulting from adherence to the turbine of the grinder or the recipient. Concerning the sieving yield, the average value was 83%, which is a quite good efficiency value for this operation, in which a sieve with a mesh size of 0.5 mm was used. The appearance of the materials produced before and after sieving, and the remains of the sieving are shown in Fig. 11.

Considering the above yields, it is possible to obtain from 1 Kg of frozen DB, after freeze-drying, grinding, and sieving, approximately 230 g of DB powder. Moreover, considering the extraction yield of DB from the frames, as previously mentioned, 1 Kg of frame produced 87,5 g of DB powder.

3.5. Physicochemical properties of DB powders

Having in consideration the previous results for the extraction and

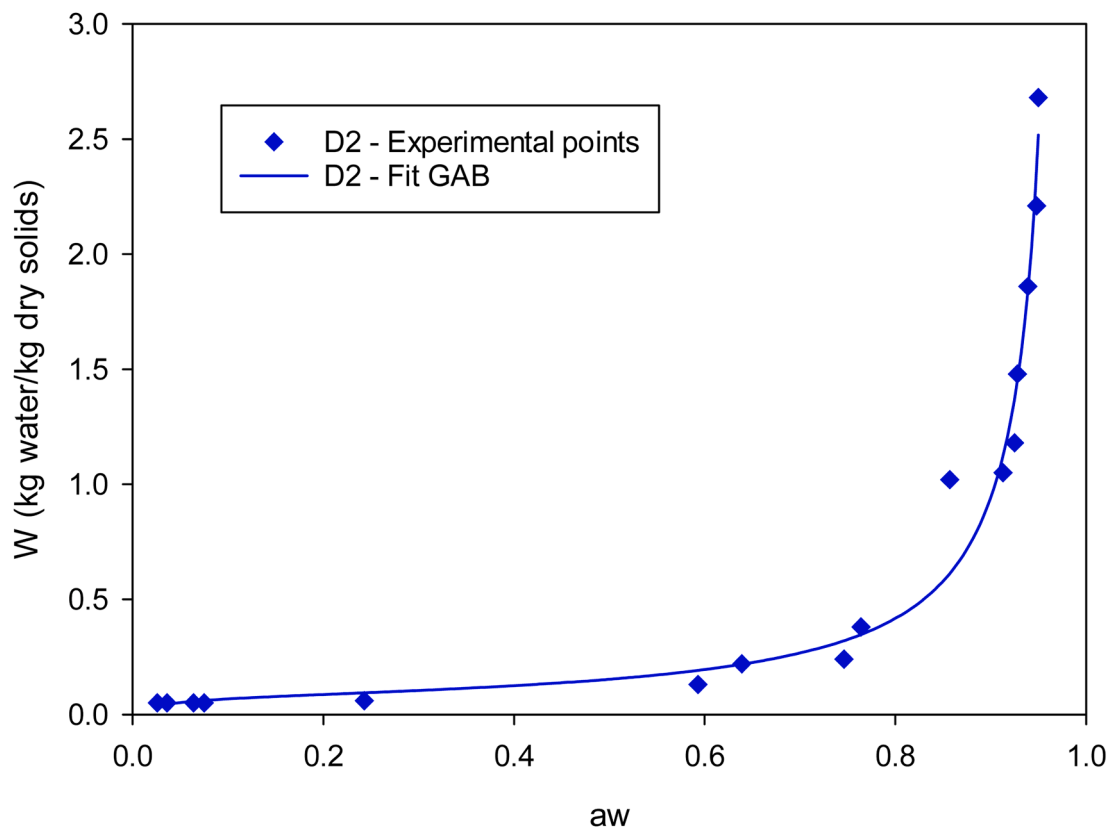
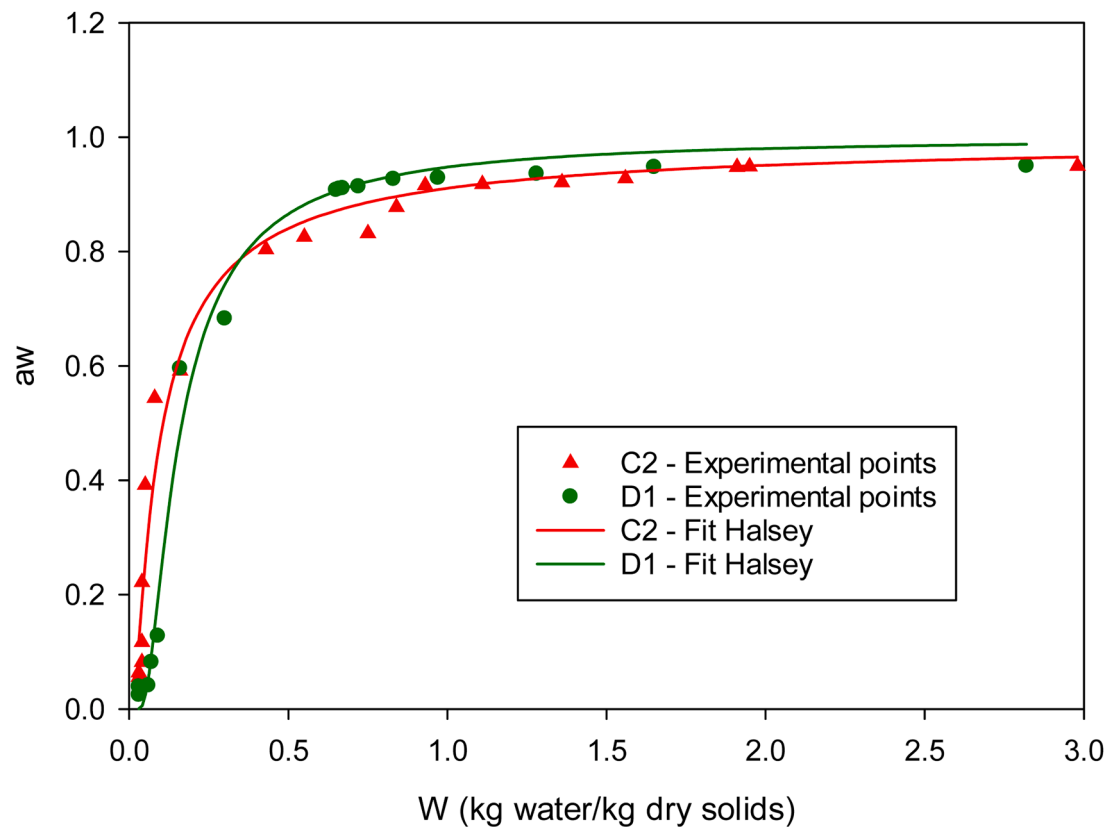


Fig. 10. Fitting of the desorption isotherms for the different extraction methods used to obtain the DB.

Table 7

Efficiency of powder production operations (freeze-drying, grinding and sieving) for DB extracted on a cold surface (D1).

Sample	Frozen drones [g]	Lyophilized drones [g]	Lyophilized yield [%]	After Grinding [g]	Grinding yield [%]	After sieving [g]	Sieving yield [%]
1	254.7	61.1	24%	61.0	100%	49.0	80%
2	273.2	80.8	30%	76.7	95%	63.0	82%
3	235.0	92.0	39%	90.8	99%	120.5	87%
4	246.0	59.6	24%	59.6	100%	48.6	82%
5	348.7	81.4	23%	80.9	99%	69.7	86%
Mean	271.5	75.0	28%	73.8	99%	63.2	83%
Sd	45.4	14.1		13.4		15.6	



Fig. 11. Appearance of the powder produced by the extraction method of frozen DB on a cold surface (D1) before and after sieving.

freeze-drying processes, for the physicochemical characterization of the powders it was not considered the extraction method in a cold room (method D2) since the extraction environment conditions for the operator were very hard, and the DB presented similar properties as the ones extracted on a cold surface (D1). Thus, just the DB powders obtained from the extraction methods on cold surface (D1) and boiling temperature (C2) were analysed.

One important issue that can limit the use of the powder in gastronomic application is the colour properties. Fig. 12 shows the appearance of the powders resulting from both DB extraction methods, cold surface (D1) and hot extraction (C2). The appearance of the powders are very different, the one produced from the cold surface being lighter and less yellow than that produced from boiling extraction.

The powders obtained from the DB extracted with the boiling (C2) and cold surface (D1) processes were evaluated for colour parameters, considering two batches for each extraction method. From the results (Fig. 13), it can be observed that for lightness, L, considerably higher values were obtained for both samples (A and B) of the powder resulting from cold extraction. Furthermore, powder obtained from the cold

extraction method of DB was whiter (lighter) than the hot-extracted powder, which was darker, meaning that the L coordinate presented lower values. This is corroborated by the images presented in Fig. 12. Concerning colour coordinate a*, powders from cold extraction (samples A and B) presented negative values, which indicates green tonalities. On the contrary, the powder samples from boiling extraction (C and D) always showed positive values of a* and indicative of red colouration. The colour coordinate b* for both powder sample presented positive values, meaning that the powders were yellow. It can also be seen that there were differences between the samples taken from different batches, both for the hot DB extraction process (C2) as well as for the cold surface extraction process (D1). Thus, it is possible to conclude that the DB extraction method and the DB batches can really influence the resulting edible powder, thus limiting its application and compromising the organoleptic quality and possible gastronomic utilization.

The results from the centesimal composition can be seen in Table 8. It can be observed that the DB and the DB powders are abundant sources of protein, fat, and carbohydrates, although poor in fibre and ash. These results are aligned with the results obtained by other authors for bee and drone brood (Finke, 2005; Rutka et al., 2021). The DB powder produced using the cold extraction method (D1) had a similar composition to DB when taken directly from the frame when it has not yet undergone the freeze-drying process. However, the powder obtained by the hot extraction method (C2) showed notable differences, particularly in the fat content, probably due to the remains of wax and other materials that were also extracted. Furthermore, powder produced by the boiling extraction method (C2) presented a lower content of the other components comparing with the powder produced by the cold extraction (D1), which could be due of the lixiviation of these compounds when the DB were immersed in the boiling water, and due to the presence of other materials, like wax, that may be poorer in these components compared to DB powder.

4. Conclusions

In this study different extraction methods of DB were tested. Three extraction methods were considered for further study: hot extraction

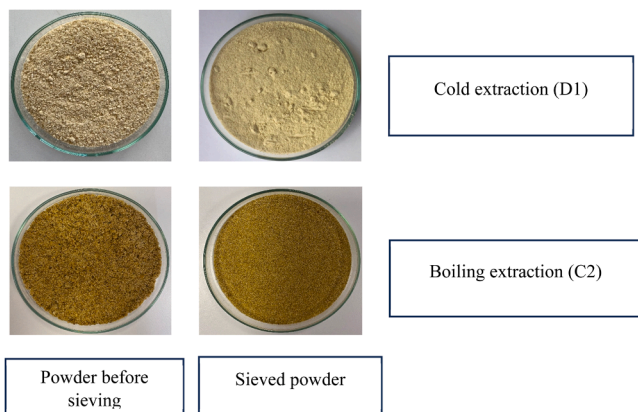


Fig. 12. Appearance before and after sieving resulting from cold and hot extraction of DB.

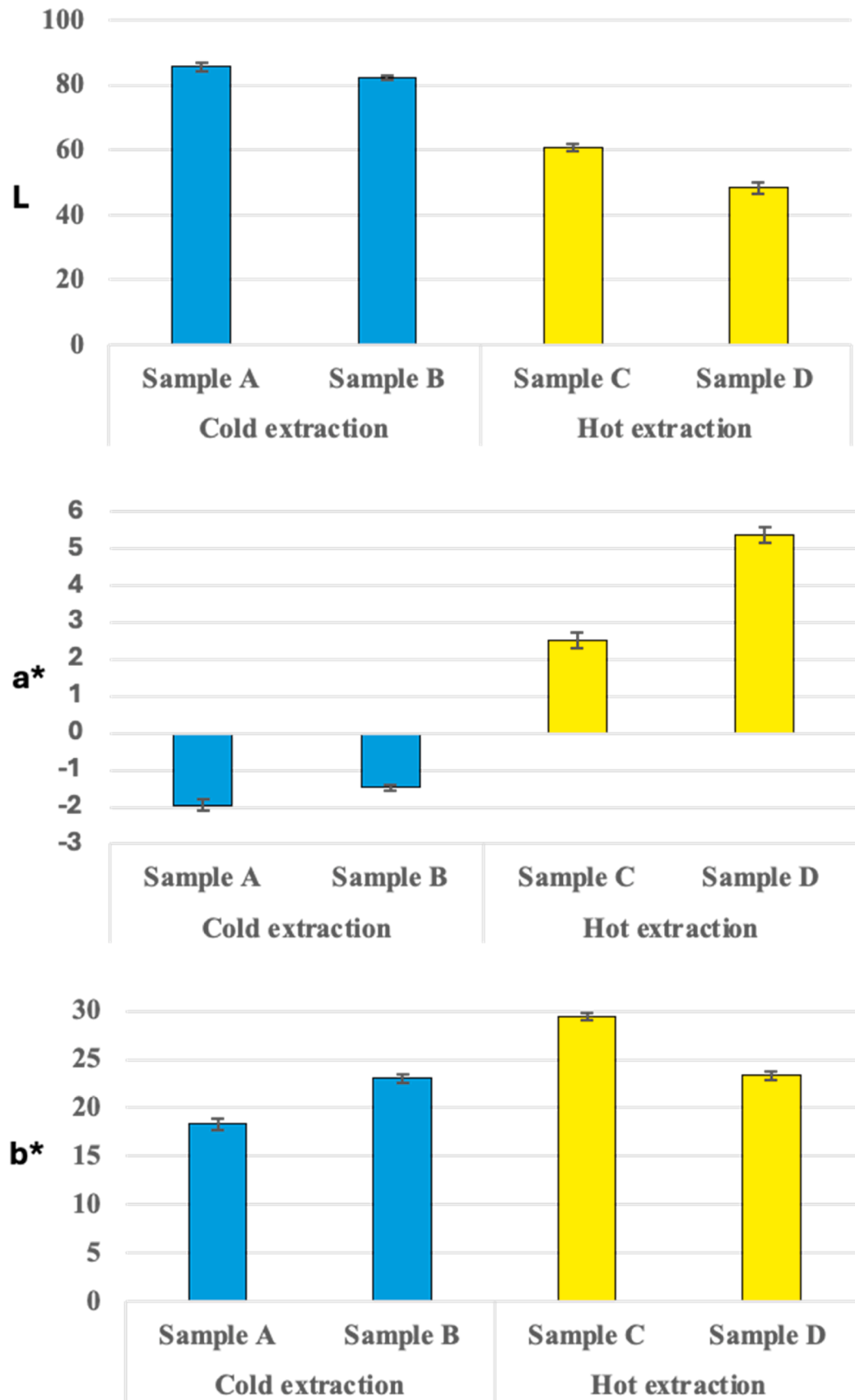


Fig. 13. Colour parameters of DB extracted by boiling and cold surface methods.

Table 8

Proximate composition of DB powder (g/100 g of dry basis).

Sample	Ash	Protein	Fat	Total sugars	Fibre
DB	3.26 ±0.03 ^b	37.32 ±0.74 ^b	25.24 ±0.23 ^a	15.80 ±0.01 ^c	4.43 ±0.02 ^b
Powder cold extraction (D1)	3.18 ±0.03 ^b	38.68 ±0.05 ^c	25.73 ±0.03 ^b	10.80 ±0.05 ^b	4.42 ±0.22 ^b
Powder boiling extraction (C2)	2.25 ±0.04 ^a	27.86 ±0.19 ^a	44.89 ±0.04 ^c	2.92 ±0.13 ^a	3.93 ±0.17 ^a

Values are the mean±Sd. Different superscript letters in the same column indicate statistically different values, according to ANOVA with Post-Hoc Tukey test ($p < 0.05$).

(C2), cold extraction on a cold surface (D1) and in a cold room (D2). A prototype piece of equipment was also developed for cold extraction on a cold surface. Concerning the extraction methods, boiling extraction showed the highest extraction efficiency, compared to extraction inside a cold room or over a cold surface, mainly because of extraction of other materials, like wax. Thus, DB obtained by boiling method had a greater amount of residues, meaning that the separation was not so clean, in opposition of the DB extracted at cold conditions which more pure.

After obtaining the DB, results showed that the drying by lyophilization is recommended, because it preserved in a better way the appearance and composition of the DB.

Concerning the colour parameters, the results showed that the DB with boiling extraction was darker than that resulting from cold extraction. Also, it was observed that the colour was not uniform and depended on the batch. This could limit the gastronomic application of the powders if the colour is a determinant factor for its acceptance and quality.

DB powders are rich in protein, fat, and carbohydrates. However, the extraction method influences the composition of the powder, mainly the fat content, which was higher in the DB powder extracted with boiling water.

This work showed that DB can be efficiently produced, extracted and processed to proportionate a valued added product (or by-product) of the beekeeping sector, and could be applied in food sector after being authorised for consumption by legal authorities. However, it is important to notice that the production of the DB and the techniques used for their removal from the frames and all the operations until production of the powder are factors that can impact the yield as well as the quality of the powders, depending on the final utilization envisaged.

Ethics not applicable

- no human subjects involved This word does report any information that concerns human subjects, and therefore, no ethical issues apply.

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CRediT authorship contribution statement

João C. Gonçalves: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Beatriz Vouga:** Investigation. **Cristina A. Costa:** Writing – review & editing, Methodology, Investigation,

Conceptualization. **Fernando Gonçalves:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Catarina Coelho:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Raquel P.F. Guiné:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Paula M.R. Correia:** Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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