

Effect of different drying techniques on the nutritional properties of *Hermetia illucens* **larvae**

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ABSTRACT

By 2050, the UN predicts a world population of 9.7 billion. To meet the protein needs of this population, food production will have to increase by 70% and there are several challenges to be overcome to reach this goal. Insects have proven to be a sustainable alternative to meet this protein demand and the black soldier fly (BSF, *Hermetia illucens* L.) stands out due to its high protein value and ease of rearing. Also, besides being a source of lipids and fatty acids, its nutritional value includes chitin, that acts as a fiber. We analyzed the influence of different drying techniques - fixed bed drying (FB), oven drying (OD), wind tunnel drying (WT) under different temperatures (50; 55 and 60ºC); sun drying (SUN) and microwave drying (MW) at different powers (20 and 50%) - on the final moisture content and on the nutritional value (protein, lipid and ash content) of black soldier fly larvae. A randomised block design (RBD) was used in a factorial scheme $(3 \times 3) + 3$ additional controls. Data were subjected to variance analysis and Tukey's test and Dunnett's test were used for comparisons of means. Significant differences were found for lipid content after the application of the drying techniques. The results showed that higher temperatures and conditions tend to favor an increase in lipid content in the dry material. FB drying at 55ºC returned the highest result (35.24%), statistically comparable to FB-60 (35.00%), MW-50 (34.77%) and BV-60 (34.61%) . In contrast, proteins returned higher values in less strenuous conditions. WT at 50ºC (36.42%) and oven drying at the 3 temperatures surveyed (35.71%; 36.10% and 35.49%) provided the highest results for protein. These studies will contribute to the establishment of an efficient means of drying for BSF larvae. We also present a survey on the pre-treatments that should precede drying to ensure the best food safety.

Keywords: *Hermetia illucens,* Pretreatment*,* Drying*,* Insects*,* Food safety.

1 INTRODUCTION

By 2050, the UN predicts a world population of 9.7 billion inhabitants (UNFPA, 2019). With this growing population, and greater wealth, a 68% increase in demand for animal protein is estimated to occur by this date (Massé et al., 2020). Food production will have to increase by 70% in order to cover the food needs of the world's livestock (poultry, pigs and cattle) (Alexandratos and Bruinsma, 2012; Konuma, 2018).

Humanity will have to find solutions for the problems arising from this growing demand. Livestock production already accounts for 70% of all use of agricultural land (Mozhui et al., 2020) and its increase would generate even more deforestation, with inevitable climatic consequences. In

addition, livestock farming is responsible for 16% of global greenhouse gas emissions, more than all modes of transport combined (Bernard, 2021). There is also a growing tendency to allocate part of agricultural production, especially corn and sugar cane, to biofuel production, reducing the supply of these products for food use. The water used for irrigation and herd maintenance is another critical factor: it is estimated that 1 kg of grain requires 1,000 litres of water, 1 kg of chicken requires 2,300 litres, 1 kg of beef requires 22,000 litres of water, and this can reach 44,000 litres (Aravind et al., 2019).

Some solutions to these problems have already been pointed out (van Huis et al., 2015): reducing meat consumption, increasing the efficiency of the food chain "from field to fork", reducing losses and changing eating habits to give preference to products that require less land to produce.

In this scenario, the use of insects for both animal feed and direct food should be considered a viable strategy that can largely solve the problems listed above (van Huis et al., 2015). The introduction of insects into the food chain will bring at least 5 immediate environmental benefits compared to current livestock: (1) lower greenhouse gas emissions (van Huis and Oonincx, 2017); (2) smaller area for production of the same energy content (Bosch et al., 2019; Cadinu et al., 2020); (3) insects have high feed conversion efficiency, defined as the amount of food required to produce 1 kg of edible body weight (van Huis et al., 2015), are twice as efficient in food conversion as poultry, four times more than pigs and twelve times more efficient than cattle (Oonincx et al., 2015) and, when incorporated into rations, they will allow grains and plants to be redirected to human food (Cisse , 2019); (4) insects do not need much more water than that which is contained in their food (Cadinu et al., 2020); (5) insects can be generated from waste and even manure, allowing the recovery of this waste and decreasing its harmful effects on the environment (Dussault, 2017).

Although insects have been part of human diet for thousands of years, studies on the subject need to be deepened before they can be used on a large scale as feed or direct food. There are basically 4 main categories of risks associated with insect consumption as food (Cappelli et al., 2020; Testa et al., 2017): chemical, related to contaminants produced by the insect or accumulated by it through its feeding; physical, although rare it may occur due to the rigid nature of some insects; allergens, since insects have the same allergens as crustaceans and arachnids (spiders, scorpions and mites) and are very close to molluscs, they can give rise to cross reactions or allergies; and finally, microbiological risks, which although few, cannot be disregarded. If all the sanitary care applied to food production is also applied to insect production, and given the phylogenetic distance between insects and higher animals, these risks are minimised (van Huis, 2021; van Huis et al., 2014).

In addition to further studies on the problems cited above, other studies concerning insect production should be carried out, such as the effect of each stage of the insect preparation process (harvesting, slaughtering, drying, separation etc.) on their nutritional constituents and the effect of their introduction into animal nutrition and human diet.

2 THE BLACK SOLDIER FLY (*HERMETIA ILLUCENS* **- LINNAEUS, 1758)**

The *Insecta* class represents over 80% of the animal kingdom and contains more specimens than the species of all other classes combined. However, although there are thousands of species of insects, not all of them lend themselves to food and mass cultivation. Life cycle control, rate of conversion of food into protein, nutritional value, resistance to stress, growth rate and nutritional requirements are some factors to be considered when choosing the species to be cultivated (Hénaultethier et al., 2015).

Among the various insect specimens already studied, the larvae of the black soldier fly (BSF) have stood out as one of the most promising for meeting the listed requirements (van Huis et al., 2014). It has also been, along with *Tenebrio molitor*, one of the most studied insects of late (van Huis, 2020).

The BSF is highly resilient and can live in adverse conditions, feeding on a wide variety of foods, which makes its cultivation quite simple. The BSF larvae are very effective in processing waste organic matter. For every 2 kg of food waste, BSF can produce 1 kg of larvae (Dussault, 2017). In a context where 1.3 billion tons of organic waste are generated globally per year (Ananno et al., 2021), the cultivation of BSF larvae can enable the reintroduction of this waste into the food chain by decreasing the losses and pollution caused by them. Also, BSF is highly nutritious. Its nutrient composition is strongly dependent on its diet and its dry biomass can contain up to 50% protein, 35% fat and 20% carbohydrates depending on its diet (Surendra et al., 2020; Shumo et al., 2019) (Nyangena et al., 2020). The amino acid pattern changes little with diet; however, lipid composition can be altered by different larval diets and, depending on the intended purpose (animal feed or industrial use), it is possible to "design" the desired larval fatty acid composition (Müller et al., 2017). Larvae are also rich in mineral salts in comparison to several other insects (Jansen, 2018).

The BSF, as well as not being considered a pest as the adult individual has no mouth, does not feed and is not attracted to human habitats or food, thus living its life separately, also acts in the control of other insects. By colonising poultry and pig manure it causes a reduction of more than 90% in the population of houseflies (Jansen, 2018). Also, due to the ability of BSF larvae to rapidly process organic matter, the risk of microbial growth in these food wastes is decreased (Gold et al., 2018).

3 PROCESSING

Insect processing begins with harvesting and should end in obtaining a safe and stable product from a microbiological and physicochemical point of view (Larouche et al., 2019). The choice of BFL processing techniques to guarantee their suitability for consumption will be a determining factor in obtaining a healthy product that can be incorporated into the food chain (van Huis et al., 2013; Purschke et al., 2018).

BSF larvae in their fresh state have a high water content (65%), a large and varied number of microorganisms, several nutrients, such as proteins and lipids, as well as a pH close to neutral, making them highly perishable (Wynants et al., 2019). Therefore, before being used as food or feed, they must be processed to ensure three important factors: quality, safety and conservation. After processing, the product obtained should have proven nutritional quality, adequate biological and chemical contaminant content and should be dry and stable for storage (Cisse, 2019). The techniques used in processing should also exhibit sustainability, be feasible on an industrial scale and be affordable (Vandeweyer et al., 2017).

Processing steps will differ according to the destination of the product and may include postharvest processing, blanching, slaughtering, decontamination, drying, grinding and macronutrient extraction (i.e., protein, lipids and chitin).

3.1 FASTING

Due to the impossibility of removing the gastrointestinal tract from the insect, it usually becomes part of the finished product. To reduce the organic matter contained in the intestines of the larvae, and its consequent microbial load, it is recommended that the larvae are left to fast for a period to empty the gastrointestinal tract (van Huis et al., 2013). This period should be sufficient for such emptying, but not too long so as to compromise the energy reserve of the insect (Barragan-Fonseca et al., 2018). Aperiod of 24 hours, followed by washing of the larvae, seems to be the consensus; however, the result of this process on the microbiota of the larvae varies in different studies (Wynants et al., 2017; Mancini et al., 2019).

3.2 SLAUGHTERING

Slaughtering is an essential step in insect processing as it affects the nutritional quality, microbial safety, color and flavor of the product (Farina, 2017). As with any animal production, slaughtering should be fast and efficient, should help to reduce the microbial load and maintain the nutritional quality of the product.

Heat treatment, asphyxiation, mechanical interruption and freezing are the most common procedures for slaughtering BSF larvae (Larouche et al., 2019). Studies indicate that insects have a sensory response to pain; (Gjerris et al., 2016) therefore, ethical aspects of animal welfare need to be considered and slaughter methods should minimise suffering (van Huis et al., 2013). Insects are pecilothermal, so killing by cold reduces their metabolism, preventing any potential pain or suffering. However enzymatic activity remains active after freezing and may induce lipid degradation and polyphenol oxidation (Caligiani et al., 2019; Leni et al., 2019). Slaughtering by heat treatment (blanching in boiling water for a short period) can solve this problem - it is fast, reduces larval moisture,

minimises lipid oxidation, microbial contamination and color changes (Larouche et al., 2019) . The use of CO2 renders the larvae unconscious and, when applied before heat treatment, solves the ethical problem (Zhen et al., 2020).

3.3 BLANCHING

In BSF larvae, the enzyme responsible for lipolysis was shown to be highly active, and continued to promote lipid spoilage even under storage at -20 °C (Caligiani et al., 2019). In larvae, blanching has the aim of inactivating these enzymes and reducing the microbial load before storage or further processing (Vandeweyer et al., 2017). This prevents these enzymes from causing alteration in nutrients and sensory qualities due to oxidation (Bazinet and Castaign, 2011) and helps maintain the colour and nutritional quality of the insect (Leni et al., 2019). Blanching also has other previously documented advantages: increasing pH by 0.5 units for at least 48 h, giving greater stability to the product compared to its in natura form(Tonneijck-Srpová et al., 2019) and reducing drying time, probably by compromising the integrity of the wax-coated cuticle that reduces dehydration of larvae (Saucier et al., 2021). As for time, 40s in boiling water is effective in reducing most microorganisms (Vandeweyer et al., 2017). Blanching for longer time may cause leaching of soluble substances such as minerals, carbohydrates, proteins and soluble vitamins (Bazinet and Castaign, 2011).

3.4 DRYING

Drying, combined with the previous treatments, is extremely important, and has direct influence on the nutritional value of BSF (HUANG et al., 2019) and its physicochemical aspects. The drying process consists of removing the water contained in a specific product to an extent that it is transformed into a dry product with low residual moisture (Badaoui et al., 2019), and is a traditional food preservation method widely applied to fruits, vegetables and meat products (Azzollini et al., 2016) to inhibit or delay microbial growth, enzymatic activity and browning reactions (Kröncke et al., 2018). Microbial growth is highly water dependent and microorganisms exhibit slow growth when water activity (AW) is low, the vast majority ceasing their growth at AW<0.65 (Grabowski and Klein, 2017).

Microwave drying and hot air drying are some of the techniques that have been used to dry and increase the shelf life of BSF larvae. The hot air drying process consists of submitting a moist product to a hot air stream (at least 10°C above room temperature) with controlled temperature, humidity and speed (Grabowski et al., 2003). In this situation there is a temperature and partial water pressure difference between the product and the air, causing not only heat transfer by convection, due to the temperature gradient, but also water transfer due to the vapour pressure gradient between the product surface and the one in the air. This mass transfer occurs mainly by diffusion, following Fick's Law (Michelin et al., 2015; da Silva et al., 2019). Microwave drying is based on interactions between an

electromagnetic field and the chemical structure the food (Regier et al., 2017). The water molecule is bipolar and microwaves cause a realignment of the molecule causing it to rotate. This rotation produces friction and generates heat by volumetrically heating the material, allowing a higher diffusion rate and higher pressure gradient which drives moisture away from within the material (Kumar and Karim, 2019).

Table 1 shows a comparison between the equipment used in hot air and microwave drying. Microwave drying is a fast process, whereas hot air drying dehydration is slower, which results in different products in terms of shape, color and apparent density. The drying method also influences the nutritional values of the dried larvae by affecting the protein structures of the BSF. Larvae dried by traditional methods, such as drying using hot air up to 60°C, show better digestibility and a higher rate of digestible indispensable amino acids compared to microwave drying; however, both techniques show values that meet FAO requirements for indispensable amino acids (Huang et al., 2019; Kim et al., 2021). Drying temperature is also an important factor to consider, as drying insects at high temperatures causes browning, marked shrinkage and loss of flavor and nutritional value (Purschke et al., 2018; Parniakov et al., 2021). Drying at lower temperatures for a longer period of time also has a negative impact on product quality (Wade and Hoelle, 2020). Drying techniques also differ from an economic point of view. Table 1 shows that for small scale production MW drying and hot air drying require low investment; however, hot air drying, being a passive process and working with larger batches, demands less labour and has a lower maintenance cost (Dortmans et al., 2021). At an industrial scale some parameters tend to change. MW equipment is very expensive and hot air drying is labour intensive. Solar drying presents itself as an option. It has low implementation and maintenance costs; however it depends on the periodic character of solar radiation (Hernández-Álvarez et al., 2021) and has a greater ease of contamination (Yi et al., 2020).

Param eter	Microwave	Hot Air
Aspect	Crispy, puffy	Hard, rigid
Colour	Yellowish	Dark brown
Density	$110 - 130$ g/l	$220 - 230$ g/l
Energy Source	Electricity	Gas
Heating form	Electromagnetic waves	Hot air
Temperature	Max. 180 °C	Max. 65° C
Batch Size	0.25 kg	30 kg
Drying time	15 minutes	24 hours
Throughput	$1,0 \text{ kg/h}$	$1,3 \text{ kg/h}$
Space usage	0.4 m2	2 m2
Energy consumption / kg dried larvae	3.7 kWh	10,9 kWh1
Investment	Very low	Low
Labour	High	Low

Table 1. Parameters associated to the hot air and microwave drying process (adap ted from DORTMANS et al., 2021)

¹Energy used is shown kWh: 1 kg gas equals $13.6Wh$

Taking into consideration quantity and objective, the drying technique and its specific conditions must be chosen in a way that guarantees a high quality product with high energy efficiency and low environmental impact which results in stable and sustainable dried food (Calín-Sánchez et al., 2020).

4 MATERIALS AND METHODS

Randomised block design (RBD) in a factorial scheme $(3 \times 3) + 3$ additional controls was used. Each drying procedure and their respective laboratory analyses were performed in triplicate. The 20 day-old larvae in the pre-pupa stage were supplied by *Verdear Ambiental Ltda* and the trials were conducted at the *Federal Institute of the North of Minas Gerais* (IFNMG) and *Federal University of Minas Gerais* (UFMG) facilities in Montes Claros-MG. After being washed in running water and rinsed with deionised water, the larvae were killed by freezing at -20ºC and kept at this temperature until the moment of use. Prior to the dehydration process, 50g samples of larvae were thawed, blanched for 40 seconds in boiling water at a larva-water ratio of 1:12 (wt/wt) to prevent water temperature drop (Purschke et al., 2018) and immediately cooled in deionised water at room temperature. The larvae were then dried on DURX® towels, weighed, distributed in a thin layer and subjected to dehydration with weighings every 15 minutes.

For dehydration in an oven, an equipment with forced circulation of the brand Nova Ética model 410/3NDRE was used. The dehydration in Fixed Bed (radius of 5.5 cm) and Wind Tunnel (40 x 11 cm) was conducted in an adapted UP Control educational module. The previous dryings were done at temperatures of 50, 55 and 60ºC. In the Fixed Bed the average wind speed used was 6.09 m/s and in the Wind Tunnel 4.11 m/s. The parts used in the adaptations were modelled and printed in a 3D printer. For microwave drying, an Eletrolux MA30S (2480 MHz frequency and 1.35 kW power) model was used, adjusted to 20 and 50% power. The solar heater was built in a stainless steel box (16 X 24.5 X 7 cm) which had been painted matte black internally, covered externally with styrofoam and covered by a transparent glass plate. The equipment was positioned with an inclination of 32º in relation to the ground. The larvae were positioned at an intermediate height in the box on a steel screen and openings at the bottom and top allowed air to circulate through the sample. With the help of a rod placed perpendicularly to the box, and observing its shade, the equipment was repositioned every 30 minutes, following the movement of the sun for better incidence of the sun's rays. In all the processes, the drying was carried out until the difference in the weight of the sample between two weighings was less than $0.5%$.

Protein analysis followed the AOAC 928.08 method using a factor of 6.25 for conversion of N into protein. Dry matter followed AOAC method 950.46 and for ash determination AOAC method 923.03 was used. Analysis of ether extract followed AOAC method 960.39 (AOAC, 2002).

5 STATISTICAL ANALYSIS

Statistical analyses were performed in RStudio 2021.09.1 (2021) software (RStudio, PBC). The data were submitted to analysis of variance and for comparisons of means Tukey's test and Dunnett's test were used. P-values lower than 0.05 were considered statistically significant.

6 RESULTS AND DISCUSSION

Analysis of the in natura larvae showed 36.32%(DM) for protein, 30.30%(DM) for lipids, 19.31%(DM) for ash and 62.30% for moisture. These results are consistent with those found by other authors (Surendra et al., 2020; Shumo et al., 2019). The results of the physicochemical analyses of the larvae after the drying treatments are presented in Tables 2 and 3. In general, after drying the larvae, there was a reduction in the protein and ash contents and an increase in the lipid content when compared to the in natura larvae. Different results for nutritional values after drying of the larvae are explained by changes in the biological matrix. The concentration of these values in the matrix is the result of a complex combination of factors, such as proteolysis and enzyme activity, and drying promotes new molecular arrangements in the matrix, which change the protein and lipid content (Kröncke et al., 2018). Solubilisation of proteins and mineral salts during blanching and denaturation and browning reactions may explain the reductions in these components (Akonor et al., 2016; Lenaerts et al., 2018).The drying technique that returned the highest result for protein was WT at 50°C (36.42%), statistically equal to oven drying at the 3 temperatures tested (35.71%; 36.10% and 35.49%). Ethereal extract, which we can relate to lipid content, showed the best result for FB at 55ºC (35.24%), statistically comparable to FB-60 (35.00%), MW-50 (34.77%) and WT-60 (34.61%). Figure 1 shows the drying kinetics curves (X/X0 vs time) for the treatments involving hot air. The drying kinetics was plotted by determining the relative water content X/X0 (where X is the water content after drying and X0 is the initial water content) over the drying time. The highest results for ethereal extract appear in the processes with shorter drying times and higher temperatures, suggesting that these conditions tend to favour the increase of lipid content in the dried material.

Table 2. Nutritional properties of black soldier fly larvae after drying processes

Averages followed by different letters, upper case in the column (equipment) and lower case in the row (temperatures), differed by the Tukey test (p<0.05). Averages followed by $*$, + and \circ differ from the control treatments by the Dunnett test (p<0.05). Results referring to dry matter.

Table 3. Nutritional properties of black soldier fly larvae after drying processes

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The ash content showed only small differences by Tukey's Test and showed no significant differences when compared to the controls by Dunnett's Test. As the ash depends on the content of minerals that are not affected by temperatures, such as those used in the trial, this small variation had already been expected and can be explained by the modifications in the biological matrix due to blanching (Mutungi et al., 2019). As for residual moisture, i.e., that which remained in the sample after the drying procedure, it was, on average, also within the intended rates (7%). Fixing an exact same moisture for the dried larvae was not possible due to the control limitations of the various drying methods. Sun drying showed the lowest value for this parameter, possibly due to the high temperatures reached (72°C) during drying. As already seen, the drying of the larvae follows Fick's Law, so higher temperatures will establish higher gradient differences facilitating evaporation. Subsequent drying at 105ºC of the previously dried samples showed values very close to and in agreement with the literature (Surendra et al., 2020; Shumo et al., 2019). An experiment that stands out from the others for its

peculiar results is MW-50% which, in addition to presenting ash content below the other experiments, presented a value for desiccation at 105°C, far above the others despite its residual moisture, which, although low, did not deviate considerably from the average. As the calculation of the other constituents is done on a dry basis, the high loss by desiccation, due to the rearrangement of the biological matrix, may have contributed to the concentration of these elements (Wasswa et al., 2021).

Taking into account that the two most important components to be recovered after drying are protein and lipid, and in order to identify the technique with the best result for these two components together, the sum of these was analysed. MW at 50% power had the best result (69.35%), along with LF-60 (68.68%), LF-55% (68.17%) and TV-60 (67.07%). Previous studies have shown that microwave-dried insects tend to increase the non-digestible fraction of the protein (Kim et al., 2021; Kröncke et al., 2018), so this result should be interpreted cautiously.

Figure 2 shows the values of the Pearson correlation coefficients (r) among the variables involved in the experiment (with the exception of the microwave experiments as they involve different processes) and demonstrates that there is a correlation among temperature, drying time and residual moisture content. Lower temperatures demand longer times and return a higher residual moisture content, which is in accordance with Fick's Law. There is also a direct correlation between the residual moisture content and the protein content, which leads us to infer that milder heating has a positive effect on the protein content. This can also be seen directly in the graph where we see an indirect correlation between protein and temperature. There is also an indirect correlation between lipids and proteins of the order of -0.542 (p-Value = 0.002), indicating that the increase in content of one item implies in the reduction of the other.

Figure 1. The kinetics curve the black soldier fly larvae under different drying processes, where X is the water content after drying and X0 is the initial water content.. The experiments were done in triplicate $(n=3)$.

Health of Tomorrow: Innovations and Academic Research *Effect of different drying techniques on the nutritional properties of Hermetia illucens larvae*

Figure 2: Values of Pearson's Correlation Coefficients (r) for p-Value<0.05. Microwave tests are not included.

7 CONCLUSION

All the drying processes tested caused changes in the protein, fat and ash content of the BSF larvae compared to the fresh larvae. Minor differences in protein and ash content were found between dried and fresh larvae. In contrast, lipids showed the greatest variations. The assays suggest that higher temperatures and consequently faster processes increase the content of lipids as opposed to proteins. The process that provided the best result for proteins and lipids added together was microwave at 50% power. It is a fast process but results in some problems linked to the solubility of proteins. The Fixed Bed Drying, at the three temperatures tested, also stood out from the other processes for its relative speed and for preserving both lipids and proteins.

The choice of drying technique should observe the quantity and objective of the material in order to guarantee a quality dry product and sustainable processing. Despite being dependent on solar incidence, solar drying stands out from the others due to the results it obtained for both proteins and lipids, as well as for being an economical and ecologically sustainable technique requiring low investment and low maintenance costs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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