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# The Impact of Heating Treatment of Black Soldier Fly (BSF) Larvae (*Hermentia illunces*) on Nutrient Levels

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**Abstract:** Black Soldier Fly (BSF) larvae, often called maggots, are a type of fly of the Hermentia illucens species capable of degrading organic waste. These maggots are used as an alternative raw material for poultry or fish feed due to their high animal protein content. Some larval cultivation methods may use poultry manure, which may carry Salmonella sp. bacteria. This study aimed to evaluate the presence of Salmonella sp. and the nutritional content of BSF larvae that had undergone a washing and heating process. Microbiological testing showed that all samples, including the positive control, showed negative results for Salmonella sp. growth, possibly due to low inoculum concentration, suboptimal incubation period, and competition with other Enterobacteriaceae bacteria. Proximate testing showed that washing caused a decrease in nutritional content, while heating treatment, particularly with a microwave at high temperatures (100 grams, 15 minutes), increased nutrient levels and decreased water activity. Low water activity (<0.6) indicates potential for increased shelf life. These results indicate that appropriate thermal treatment can improve the microbiological safety and nutritional quality of BSF larvae as a feed ingredient.

**Keyword:** BSF larvae, Feed, Heating, Proximate, Salmonella sp

## INTRODUCTION

Insects are currently being widely researched as an alternative protein source for animal feed. Besides their nutritional profile, their availability is guaranteed and their price is competitive. Using insects to replace fish meal and soybean meal can reduce production costs in livestock farming without negatively impacting livestock growth and productivity, considering that feed costs contribute 70-80 % of total production costs (Barantan , 2021). Research on the potential of feed ingredients as a substitute for protein sources in animal feed was conducted with the aim of knowing the economic aspects and efficiency of its use (Widharto et al ., 2020; Krismaputri et al ., 2016).

Black Soldier Fly (BSF) larvae come from one of the types of flies that are included in the species Hermentia illucens. This larva is generally referred to by the community as a maggot. BSF larvae have a high protein content and include natural feed for poultry (Makkar et al., 2014). BSF larvae are one of the fly larvae that have an animal protein content of around 30-45%. The high protein content is very potential as an alternative raw material for poultry feed or fish feed. The use of insects as a source of protein has been widely studied. According to Van Huis (2013), protein sourced from insects is more economical, environmentally friendly and has an important role in nature . Insects have a high feed conversion value and can be mass produced. Insect culture can also reduce organic waste that has the potential to pollute the environment (Li et al ., 2011).

Hermentia illuncens has been studied for its ability to degrade organic waste using its larvae. BSF larvae can extract energy and nutrients from vegetable waste, food scraps, animal carcasses, and feces as food sources (Popa and Green, 2012). The role of BSF larvae in the process of degrading vegetable and fruit waste can potentially be contaminated by pathogenic bacteria. A recent scientific review by Garofalo et al. (2019) concluded that insects that can be used as feed or food ingredients are vectors of several species of microorganisms. These microorganisms can be found in their guts or on their external cuticles. These microorganism species can act as commensals, causing spoilage, or can be pathogenic to humans (Garofalo et al., 2019; Osimani and Aquilanti, 2021). Several types of pathogenic bacteria found in the waste decomposition process include Streptococcus, Escherichia, Pseudomonas, and Proteus (Khamid and Mulasari, 2012). Salmonella sp. may be found in BSF larvae, as some H. illucens larval breeding methods utilize poultry manure.

An incomplete cleaning process for BSF larvae can potentially introduce Salmonella sp. bacteria. This condition can certainly impact the safety and quality of feed derived from BSF larvae. The presence of Salmonella sp. bacteria in BSF larvae is important to understand because it can be harmful and reduce the quality of feed safety. According to the Decree of the Head of the Quarantine Agency Number 4556 of 2021, the maximum microbial contamination limit for Salmonella sp. is negative. Monitoring and evaluation are carried out through laboratory tests to detect the presence of microbes , including Salmonella sp. and Enterobacteriaceae.

Quarantine acts as a regulator for the export process of feed raw materials and feed in providing assurance of safety and quality of feed through supervision and control integrated with quarantine actions. Supervision and control are carried out through efforts to guarantee finished products that are exported in accordance with the laws and standards that apply to the safety and quality of feed materials and feed of insect origin or insect products. Traceability in supervision to guarantee the safety and quality of feed is applied by designating the place of production as an Animal Quarantine Installation (IKH).

Supervision by the quarantine is carried out periodically to ensure that feed materials or feed of insect origin or insect products are produced from production sites that have fulfilled the procedures in accordance with the applicable legislation. In addition, surveillance by Quarantine is also carried out at ports or airports that are the work area of the Agricultural Quarantine Agency. The purpose of this research is to determine the temperature and heating time of BSF (Hermentia Illucens) larvae to eliminate Salmonella sp. with minimal impact on reducing nutritional content. The results of this research can be used as a reference for the Agricultural Quarantine Agency regarding the determination of the heating method for BSF larvae to eliminate S almonella sp with minimal impact on reducing nutritional content.

## METHOD

Larvae of BSF in the adult phase obtained from the BSF larva processing industry. This research was conducted in Agricultural Quarantine Technique and Method Application Test Center (BUTTMKP) Bekasi. The media needed to bacterial growth is aquades, PCA, BPW, LB, SCB, TTB, RV, XLDA, HEA, BSA, TSIA, LIA, LDB, KCNB, MR-VP, SCB, TB, TSTB, SIM, Kovac's Reagent, BHI, Urea Broth, Malonate Broth, Phenol Red Lactose Broth, Phenol Red Sucrose Broth. The equipment used is a microwave, oven, petri dish, test tube, pipette size 1 ml, 2 ml, 5 ml, 10 ml, media bottle, scissors, inoculation needle tweezers (ose), stomacher, bunsen burner, pH meter, scales, magnetic stirrer, tube shaker (vortex), incubator, water bath, autoclave, sterile cabinet (clean bench), refrigerator, freezer. Proximate analysis was carried out in the integrated laboratory of IPB University.

**Proximate Analysis and Microbiological Testing**

This study consisted of 3 groups of adult BSF larvae, namely the control group, the group with the washing process and the washing group spiked with Salmonella sp. with a concentration equivalent to Mc Farlan 0.5 (> 1.5 x 108 CFU/ml) with a ratio of 1:10. Before heating treatment, adult BSF larvae before and after cleaning was subjected to microbiological tests and proximate. Proximate analysis includes protein, ash, fat, water content, water activity, energy from fat, and total energy. Microbiological testing to determine of Salmonella sp growth contained in BSF Larva samples was carried out referring to ISO 6579-1:2017/Amd 2020 concerning Salmonella sp contamination testing methods.

**Heating Treatment**

The heating treatment of BSF larvae follows the stages of the production flow procedure carried out at the BSF larva processing facility. Heating is carried out using two dry heating methods, the first is using an oven at a temperature variable below or equal to 100 °C , namely 65 °C, 75 °C and 100 °C for a duration of 12.5 minutes and at a temperature variable above 100 °C , namely 150 °C, 180 °C and 200 °C for a duration of 12.5 minutes. The second dry heating method is using a microwave at variable temperatures of High, Medium - High and Medium for 15 minutes, as in Figure 1.

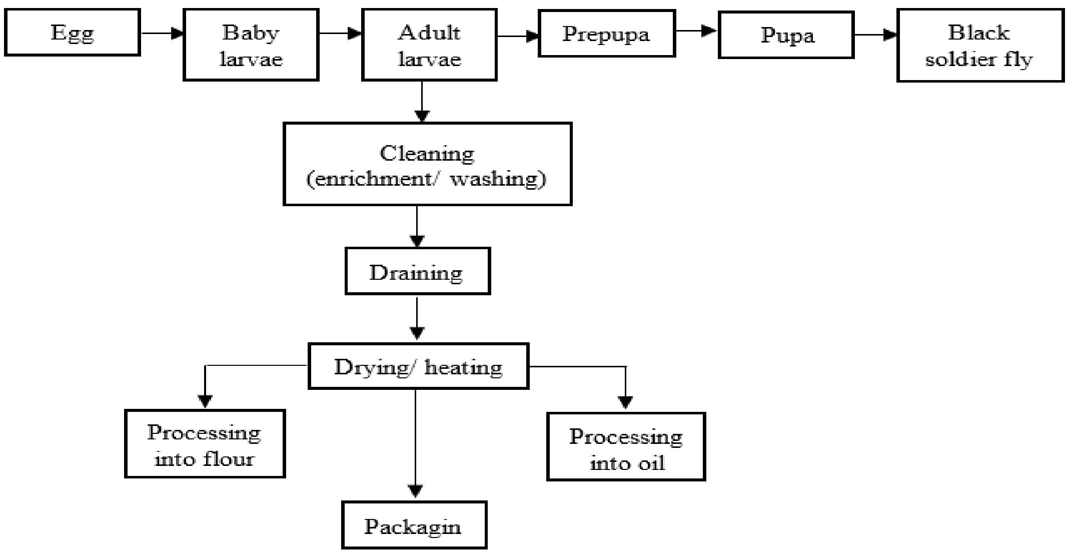


**Figure 1. Dry BSF Larvae Production Flow**

The type of oven used is the Carbolite brand with an electric heat source, a capacity of 200-400 grams for heating BSF larvae using two tray racks. The type of microwave used is the Panasonic NN-ST557M/W model with a capacity of 100-200 grams for heating BSF larvae using a 30 cm pyrex microwave plate. The dry heating treatment scheme for BSF larvae to eliminate Salmonella sp is as shown in Figure 2.

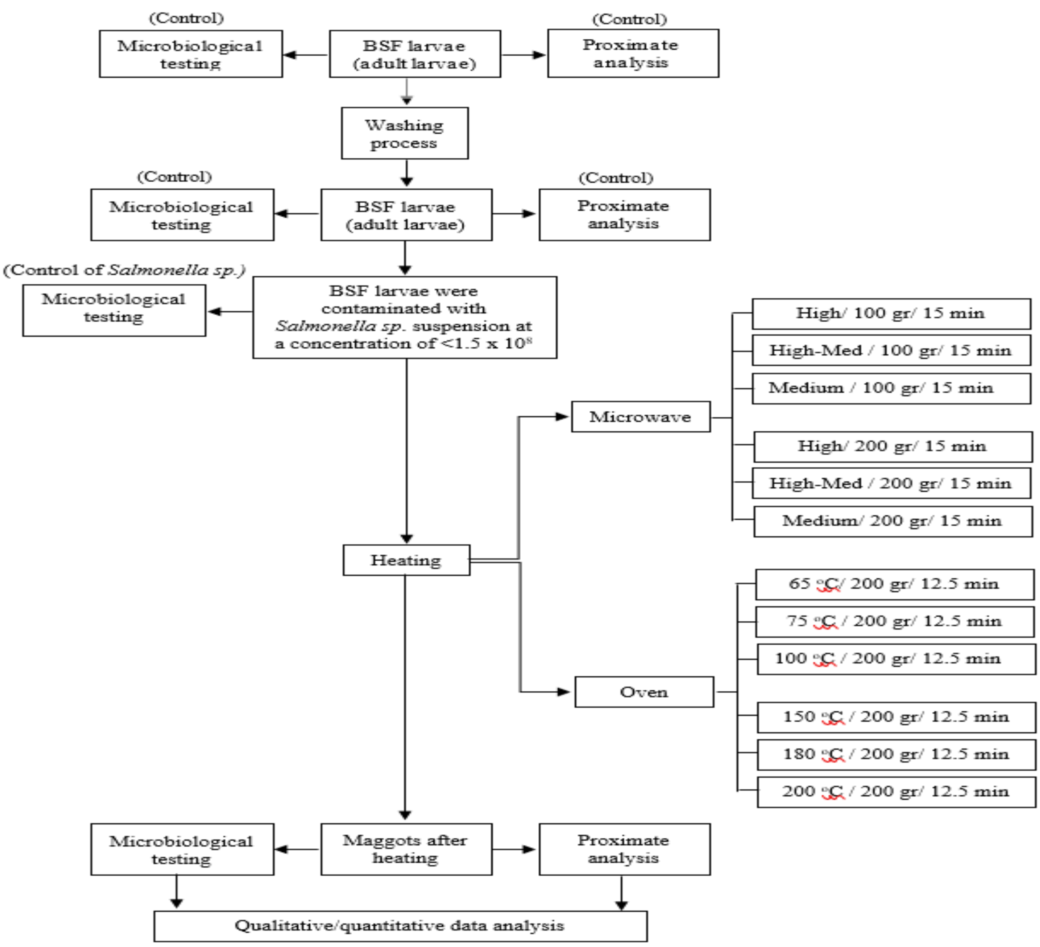


**Figure 2. Scheme of Dry Heating Treatment of BSF Larvae**

## Data Analysis

Analysis of data obtained from the results of the application test of heating treatment on BSF larvae (H. illucens) to eliminate Salmonella sp. is presented qualitatively.

## RESULT AND DISCUSSION

Testing of fresh BSF larvae in the first and second samples received aims to determine the presence of *salmonella sp* in larvae that have been cleaned and washed and continued through a proximate test to determine their nutritional content . Data from the results of microbiological testing of *salmonella sp* and nutritional content in BSF larvae can be seen in Tables 1, 2 and 3.

**Table 1. Data From *Salmonella Sp* Test Results**

| Sample delivery | Testing | Unit (gr) | Treatment | Temperature °C | Volume (gr) | Duration (min) | Result |
|---|---|---|---|---|---|---|---|
| BSF larva I | *Salm. Sp* of BSF larva | 25 | *Salm. Sp* PC | - | - | - | Negative |
| | | | | High* | 100 | 15 | Negative |
| | | | Microwave | High* | 100 | 15 | Negative |
| | | | | Hihg* | 100 | 15 | Negative |
| | | | PC of *Salm. sp* | - | - | - | Negative |
| | | | | High* | 100 | 15 | Negative |
| BSF larva II | *Salm. Sp* of BSF larva | 25 | | High-medium | 100 | 15 | Negative |
| | | | Microwave | | | | |
| | | | | Medium | 100 | 15 | Negative |
| | | | | High* | 200 | 15 | Negative |

| | | | | | |
|---|---|---|---|---|---|
| | | High-medium | 200 | 15 | Negative |
| | | Medium | 200 | 15 | Negative |
| | Oven | 65 ºC* | 200 | 12.5 | Negative |
| | | 75 ºC | 200 | 12.5 | Negative |
| | | 100 ºC | 200 | 12.5 | Negative |
| | | 150 ºC | 200 | 12.5 | Negative |
| | | 180 ºC | 200 | 12.5 | Negative |
| | | 200 ºC* | 200 | 12.5 | Negative |

Samples were subjected to proximate analysis
PC: positive control

**Table 2. Proximate test results data for BSF I larval samples**

| No | Parameter | Unit | Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | Clean larvae before washing (control) | Clean larvae after washing (control) | Microwave heating (High) | | |
| | | | | | Treament 1 | Treament 2 | Treament 3** |
| 1 | Ash content | % | 3.35 | 2.75 | 13.44 | 13.47 | 10.52 |
| 2 | Energy from fat | Kcal/100 g | 76.68 | 60.93 | 186.21 | 155.61 | 143.10 |
| 3 | Total fat content | % | 8.52 | 6.77 | 20.69 | 17.29 | 15.9 |
| 4 | Water content | % | 69.47 | 74.06 | 5.29 | 5.20 | 20.67 |
| 5 | Total energy | Kcal/100 g | 151.32 | 126.61 | 428.57 | 411.81 | 354.74 |
| 6 | Carbohydrate | % | 4.07 | 3.55 | 11.76 | 14.65 | 11.97 |
| 7 | Protein content | % | 14.59 | 12.87 | 48.83 | 49.40 | 40.94 |
| 8 | Crude fiber | % | 2.50 | 1.40 | 7.88 | 7.96 | 7.25 |
| 9 | Water activities (aW) | - | 0.929 | 0.985 | 0.495 | 0.427 | 0.85 |

Note: The calculation of nutrient content in proximate testing is based on the weight of the sample being tested.
*heating treatment with different microwave tools

**Table 3. Proximate test results data for BSF II larval samples**

| No | Parameter | Unit | Result | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Clean larvae before washing (control) | Clean larvae after washing (control) | Microwave heating (Temp, Vol, Time) | | Oven heating (Temp, Vol, Time) | |
| | | | | | High/ 100/15 | High/200/15 | 200 ºC/200/ 12.5 | 65 ºC/200/ 12.5 |
| 1 | Ash content | % | 3.28 | 2.72 | 10.42 | 6.78 | 2.29 | 2.67 |
| 2 | Energy from fat | Kcal/100 g | 33.39 | 36.09 | 226.44 | 185.94 | 122.67 | 47.25 |
| 3 | Total fat content | % | 3.71 | 4.01 | 25.16 | 20/66 | 13.63 | 5.25 |
| 4 | Water content | % | 70.54 | 75.35 | 4.65 | 37.59 | 45.10 | 76.34 |
| 5 | Total energy | Kcal/100 g | 123.35 | 107.77 | 465.52 | 325.82 | 262.59 | 110.21 |
| 6 | Carbohydrate | % | 12.66 | 4.94 | 10.87 | 2.53 | 6.48 | 3.55 |
| 7 | Protein content | % | 9.83 | 12.98 | 48.90 | 32.44 | 28.50 | 12.19 |
| 8 | Crude fiber | % | 2.12 | 1.44 | 6.78 | 2.15 | 5.56 | 2.31 |
| 9 | Water activities (aW) | - | 0.963 | 0.974 | 0.42 | 0.95 | 0.96 | 0.97 |

Note: The calculation of nutrient content in proximate testing is based on the weight of the sample being tested.

Microbiological testing on both samples showed negative results for the growth of Salmonella sp in all treatment variables. The (+) Salmonella sp control also showed negative results for the growth of Salmonella sp. There are several influencing factors and need to be corrected and retested. The influencing factors are Salmonella sp is a bacteria with growth characteristics that can be suppressed by other Enterobacteriaceae, the lack of incubation period during the contamination of Salmonella sp in BSF larvae, the uneven mixing of the Salmonella sp suspension in BSF larvae and the low concentration of the Salmonella sp suspension.

Table 2 shows that the nutritional content of BSF larvae decreased after washing and increased after heating. There was no significant difference in the data between treatments 1 and 2. Treatment 3 showed a greater difference in the data compared to treatments 1 and 2. Table 3 shows data on the increasing or decreasing nutritional content of each variable in each parameter. The data with the best nutritional content in BSF larvae was found in the high-temperature microwave heating treatment with a volume of 100 grams per heating for 15 minutes.

The results of retesting the factors that influence the negative results of salmonella sp growth in the control (+) can be seen in Table 4.

**Table 4. Data from Salmonella sp and Enterobacteriaceae test results**

| No | Parameter | Unit | Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | K1 | K2a | K2b | K3 | M1 |
| 1 | *Salmonella enteritidis* | 25 g | Negative | Negative | Negative | Negative | Negative |
| 2 | Enterobateriaceae | CFU/g | $1.4x10^6$ | $1.9x10^8$ | $7.6x10^8$ | $7.8x10^7$ | < 10 |

| No | Parameter | Unit | Result | | | | |
|---|---|---|---|---|---|---|---|
| | | | M2 | O1 | O2 | O3 | O4 |
| 1 | *Salmonella enteritidis* | 25 g | Negative | Negative | Negative | Negative | Negative |
| 2 | Enterobateriaceae | CFU/g | < 10 | < 10 | < 10 | $3.8x10^7$ | $4.0x10^9$ |

Note
K1 : Control BSF larvae without *Salmonella sp.* spike and without treatment
K2a : Control BSF larvae with *Salmonella sp*. spike and without treatment
K2b : Control BSF larvae with *Salmonella sp*. spike and without treatment
K3 : *Salmonella sp*. suspension with McFarland 3 standard (>9 x $10^8$ cfu/gr)
M1 : BSF larvae with *Salmonella sp*. spike and treated with microwave heating at high temp/100 g/15 min
M2 : BSF larvae with *Salmonella sp*. spike and treated with microwave heating at medium temp/100 g/15 min
O1 : BSF larvae with *Salmonella sp*. spike and treated with oven heating at 200 $^o$C/200 g/12.5 min
O2 : BSF larvae with *Salmonella sp*. spike and treated with oven heating at 100 $^o$C/200 g/12.5 min
O3 :BSF larvae with *Salmonella sp.* spike and treated with oven heating temp 75 $^o$C/200g/12.5 min
O4: BSF larvae with Salmonella sp. spikes and treated with oven heating temp 65 $^o$C/200g/12.5 min

Proximate testing based on dry matter (DM) on BSF larvae is shown in Table 5. Proximate testing based on dry matter (DM) shows different results for each parameter in each treatment variable . There is a decrease in the percentage of protein content and an increase in the percentage of fat content in the microwave heating variable high / 200/15. Water activity is at a level below 0.6 in the microwave heating variable high / 200/ 15.

**Table 5. Proximate test data based on dry matter (DM)**

| No | Parameter (dry basis) | Unit | Result (Temp/gr/Time) | | |
|---|---|---|---|---|---|
| | | | Control | Oven (65 oC/ 200g/ 12.5 min) | Microwave (High/100 g/ 15 min) |
| 1 | Ash content | % | 14.57 | 13.81 | 12.58 |
| 2 | Energy from fat | Kcal/100 g | 150.21 | 33.15 | 237.69 |
| 3 | Total fat content | % | 16/69 | 15.6 | 26.41 |
| 4 | Water content | % | 0 | 0 | 0 |
| 5 | Total energy | Kcal/100 g | 425.17 | 315.51 | 481.73 |
| 6 | Carbohydrate | % | 8.10 | 10.48 | 10.32 |
| 7 | Protein content | % | 60.64 | 60.11 | 50.69 |
| 8 | Crude fiber | % | 6.17 | 6.5 | 7.63 |
| 9 | Water activities (aW) | - | 0.913 | 0.929 | 0.4 |

The lower the water activity of a feed ingredient, the longer it will last and the more durable it will be. Therefore, determining the water activity of a feed ingredient is crucial for proper handling during processing and distribution. The (+) control of Salmonella sp that was grown showed negative results . This is due to several factors that influence the growth of Salmonella sp. These factors are that Salmonella sp is a bacterium with growth characteristics that can be suppressed and compete with other Enterobacteriaceae. Salmonella sp is a bacterium that grows at a temperature range of 5 °C to 47 °C with an optimum temperature of 35 to 37 °C, but some serotypes can grow at temperatures as low as 2 to 4 °C or as high as 54 °C (Gray and Fedorka Cray, 2012). The presence of Salmonella sp in washed BSF larvae could no longer be detected, so this treatment required inoculation of Salmonella sp in BSF larvae and incubation at 37 °C overnight (Looveren et al., 2021) according to the incubation period for Salmonella sp growth as previously described. The process of inoculating Salmonella sp in BSF larvae is uneven, according to Looveren et al., (2021), mixing the Salmonella sp suspension is done by inoculating it slowly little by little and then stirring it evenly using a sterile spoon . The low concentration of Salmonella sp also affects the Salmonella sp's inability to grow in BSF larvae. Salmonella sp can be transmitted through food and drink contaminated with bacteria. If the bacteria enter in large number, namely approximately 1X106 - 1X109. Looveren et al., (2021) inoculated Salmonella sp on BSF larval frass using a Salmonella sp suspension made from a mixture of 0.85 % PPS, NaCl, 0.1 % peptone which was equivalent to Mc.Farland 3 solution (9 X 10 CFU/Gram).

The heating process results in the loss of a certain percentage of the water content of BSF larvae. Fresh BSF larvae contain 70%. This water content increases after washing, resulting in a slight decrease in their nutritional value. Heating BSF larvae at high temperatures for a short period of time can increase their nutritional value.

## CONCLUSION

This treatment found that *Salmonella* sp. could be effectively eliminated during the washing process for fresh BSF larvae, thereby ensuring improved microbial safety of the product. Furthermore, microwave heating at 200°C for 15 minutes not only resulted in the highest quality product but also contributed to enhanced preservation, reduced contamination risks, and maintained desirable nutritional properties. These findings highlight the importance of combining proper washing techniques with controlled thermal processing to achieve safe, hygienic, and high-value insect-based feed or food ingredients.

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