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Cutting-Edge Insect Processing: Unlocking the Potential for Bacterial Reduction in Black Soldier Fly (*Hermetia illucens*) Protein

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ABSTRACT

Insects are rising in importance as an alternative animal protein feed source for livestock and pets. Black soldier fly larvae (*Hermetia illucens*) are one of the most common species in this alternative sector. This is based on their nutritional value, growth potential, high bioconversion ratio, and low environmental impact. The bacterial population in the larvae has been characterized but not the impact of process technology on bacterial reduction. This study focuses on the effect of insect processing on bacterial levels, from the larvae up to the protein feed generated from them. The two common processes, dry and wet processing, are compared with regard to their individual impact on product hygiene. Significant differences were observed between the technologies used for insect processing. In the dry process, a reduction of bacteria in the range of 2.63–3.41 log CFU/g was observable. In wet processing, a higher potential to reduce bacteria in the products was found, resulting in a decrease in bacterial count of 5.68 log CFU/g over the entire process. Both systems have shown different reduction potentials at different process stages. The EU Regulation 142/2011 is set as a legal benchmark in this study. Additionally, we observed a slow recontamination of the protein feed in a storage study over 14 days.

1 | Introduction

The intensification of agriculture and the high proportion of "processing losses" associated with livestock production are critically discussed in public. One major criticism raised in the context of industrial food production is that around one-third of the food produced ends up as waste during its journey from the farmer to the consumer. Along this chain, industry, trade, large-scale consumers, and private households in Germany produce about 11 million tonnes of food waste per year (FAO 2011). The German Federal Ministry of Food and Agriculture believes that "in order to efficiently break down and process biomass and make it available for downstream production processes in line with demand, the prerequisites should be created for converting by-product and residual material flows into valuable products" (BMBF 2020).

Insects, particularly black soldier fly larvae (*Hermetia illucens*, BSFL), can improve resource efficiency by utilizing by-products from the food industry, among other things, as substrate. It is

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Abbreviations: BSFL, Black soldier fly larvae; CFU, colony-forming units; EFSA, European Food Safety Authority; *ENT*, *Enterobacteriaceae*; FAO, Food and Agriculture Organization of the United Nations; FASFC, Federal Agency for the Safety of the Food Chain (Belgium); TBC, Total bacteria count; UN, United Nations.

TABLE 1 | Microorganism limit value according to regulation (EU) No. 142/2011.

Microorganism	Warning value	Limit value
Salmonella spp.	—	Not detectable in 25 g
C. perfringens	—	Not detectable in 1 g
Enterobacteriaceae	1.00 log CFU/g	2.48 log CFU/g

generally assumed that insects can be produced in a climate-friendly, space- and resource-efficient manner. BSFL has demonstrated a good feed conversion rate: under optimal conditions, 0.8 kg of insects can be produced from 1 kg of feed (FAO 2013; Makka et al. 2014; Oonincx et al. 2015).

The motivation for processing insect larvae lies in their potential benefits, particularly when utilized in industrial vertical farming systems to produce high-value meals for feed and food. Some applications focus on selling whole dried larvae, while others require disintegrating and drying the larvae to create a meal with a higher protein content. This process necessitates the economic removal of lipids, which can also add value by enabling the recovery of separate lipid and chitin fractions. Two primary industrial pathways are employed for defatting insect larvae: dry processing and wet processing. Both methods offer specific advantages depending on the application and target markets, justifying their simultaneous use. Dry processing, derived from traditional techniques like vegetable oil pressing, involves evaporating most of the water from the larvae before separation, eliminating the need for subsequent stickwater processing. In contrast, wet processing removes approximately 50% of the water through mechanical separation, enhancing energy efficiency, especially at higher processing capacities. While dry processing results in lower dissolved protein in the protein concentrate, wet processing facilitates easier batch changes and cleaning, making it particularly beneficial for food ingredient applications. Additionally, low process temperatures—critical for maintaining protein functionality and lipid color—are more easily achieved with wet processing (Sindermann et al. 2021).

The product quality arises directly from the process conditions. Differences exist between the individual thermal and mechanical process steps and their influence on the product. Maillard reactions can negatively impact product quality and represent potential process contaminants that are important for the safety of the end products. This impact can be assessed by measuring the formation of furosine during drying processes (Van Rooijen et al. 2013). However, in practice, these process steps in insect processing are often considered uncontrolled concerning their hygienic effects and are usually also insufficient for ensuring consistent microbial safety and product stability, as these steps have not yet been optimized (Belluco et al. 2013; Makka et al. 2014; Čičková et al. 2015). Various potential foodborne biological contaminants in edible insects present risks to food and feed safety. According to Vandeweyer et al. (2021), significant food safety risks are posed by bacteria such as *Staphylococcus aureus* and the *Bacillus cereus* group in edible insects. While pathogens like *Salmonella spp.* and *Campylobacter spp.* are deemed low risk, there is limited information on other biological contaminants, including viruses and prions, highlighting the need for further research.

The quality of the final product determines the required processing methods, making the choice of end product essential for the overall processing approach. This point is emphasized in the opinions of the Scientific Committee of the European Food Safety Authority (EFSA) and the Belgian Federal Agency for the Safety of the Food Chain (FASFC) (FASFC 2014; EFSA 2015; Van Looveren et al. 2022). They discuss the presence of *Clostridium perfringens* in the industrial processing of BSFL. Their study examines the process from rearing BSFL to drying the whole larvae but does not address the additional processing needed to produce commercial livestock feed materials.

Microbiological safety is essential for the use of insects or insect products in the livestock and pet sectors. The legal classification of insects as feed has been revised or adapted at the EU level in recent years. Since 2017, processed animal protein from farmed insects has been used in aquaculture according to Regulation (EU) No. 2017/893. In August 2021, insects were permitted in pig and poultry feed through the amendment of (EU) No. 2021/1372 to Regulation (EU) No. 999/2011. However, the regulatory changes in the EU are extensive and can serve as a significant limiting factor for process technology, particularly for small companies that may find it challenging to meet these stringent requirements. This means that insect protein can now be used as feed in agricultural practice and can be regarded as a viable alternative to vegetable and animal proteins (soy protein, fish meal). According to current legal practice, Regulation (EU) No. 142/2011, including the limit values described therein (Table 1), can be considered for the assessment of insects. This necessitates assessing intermediate and end products within the food and feed chain (Sudwischer and Sitzmann 2022).

The objective of this study is to evaluate the process-related differences in the hygienic potential of processed insect-based feed and to assess critical control points for product quality in industrial facilities that produce insect protein using dry or wet processing methods.

2 | Materials and Methods

2.1 | Chemicals

The following chemicals were purchased from commercial suppliers: hydrochloric acid 25% p.a. (Merck, Darmstadt, Germany), hydrochloric acid Convol Normadose 0.1 M (VWR, Darmstadt, Germany), and trifluoroacetic acid (TFA) (Fisher Scientific, Schwerte, Germany).

2.2 | Insect Samples

Insect samples were bred by the German Institute of Food Technology (DIL, Quakenbrück, Germany) and FarmInsect GmbH (Oberbergkirchen, Germany). All insects were shipped and stored frozen at −20°C. The larvae were fed with a commercial acid mixture that reduced the pH of the feed below 5. This mixture, provided by FarmInsect GmbH, consists of propionic acid, acetic acid, and formic acid; however, the exact composition is not disclosed by the company. These larvae were only used for dry processing.

2.3 | Insect Processing

The established processing methods, dry and wet processing were conducted at the IFF experimental laboratory on a small technical scale (approximately 30–50 kg/h). These methods were implemented as duplicates, both on a technical and laboratory scale. Due to the batch size constraints, the drying process was conducted in six batches, with sampling performed accordingly.

2.3.1 | Dry Process

For generating a protein meal by the dry process (Figure 1), killed insects were used (frozen; t: 4 h; T: −20°C). The insect samples were dried by using an infrared drum dryer (FS-Laborbatch; type FS 60/60–10; Kreyenborg, Germany) in 12 kg (fresh mass) batches. The drying process was operated at 90°C for 240 min to reduce the residual moisture below 6 g/100 g. The screw press (TP 04, factory No. 100032; Reinartz, Germany) was preheated before operating to a temperature above 60°C by direct infrared heating of the press housing. The insect samples were preconditioned during the process by heating in the infrared drum dryer at 45°C for 120 s. Preconditioning has a positive effect on protein coagulation and oil viscosity. This treatment should achieve large physical differences between the press cake (change in structure by protein coagulation) and the liquid fraction (change in viscosity) to obtain the highest possible yield of oil and de-oiled press cake. The BSFL were continuously pressed in the screw press until oil leaked off the case, while the press cake (protein meal) left the de-oiling section through a 4-mm perforated disc. The steady state of the machinery was reached after 15 min by constant mass flow (25 kg/h). The mean residence time in the press was approx. 300 s.

2.3.2 | Wet Process—Laboratory Scale

In the wet process (Figure 2), insect samples were used for laboratory-scale experiments (insect sample mass lab scale: 75 g). Blanching was performed for 3 min at 100°C. Before fat separation, the insects were minced using a mincer (FW1100, hole diameter 3 mm; Beeketal, Germany). The ground insects were then mixed with 0.01 M hydrochloric acid for fat digestion ($V_{\text{lab}} = 150$ mL). This process ran for 60 min after the suspension reached 60°C, and an end temperature of 100°C was achieved after approximately 8 min. The digestion on the lab scale was carried out in an Erlenmeyer flask, which served as the reactor for the fat-melting process. For heating in the laboratory scale, a silicone oil bath was used. Following this, the hot suspension was separated using a heated (60°C) lab centrifuge (Rotina 380 R, Hettich, Germany). The separation process was operated at 60°C. After separation, the solid phase was dried using an infrared drum dryer (FS-Laborbatch; type FS 60/60–10; Kreyenborg, Germany) at 80°C for 180 min (residual moisture < 14 g/100 g).

2.3.3 | Wet Process—Technical Scale

For the technical process (50 L batches), BSFL insect samples (insect sample mass: 25 kg), killed by freezing (t: 4 h; T: −20°C), were used. Before fat separation, the insects were minced using a mincer (FW1100, hole diameter 3 mm; Beeketal, Germany). The ground insects were then mixed with 0.01 M hydrochloric

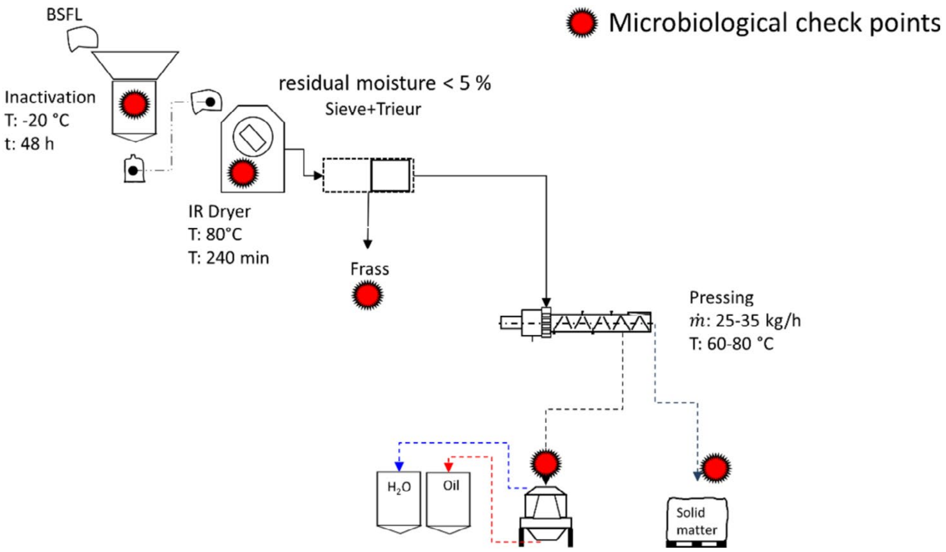

FIGURE 1 | Process diagram of insect dry process.

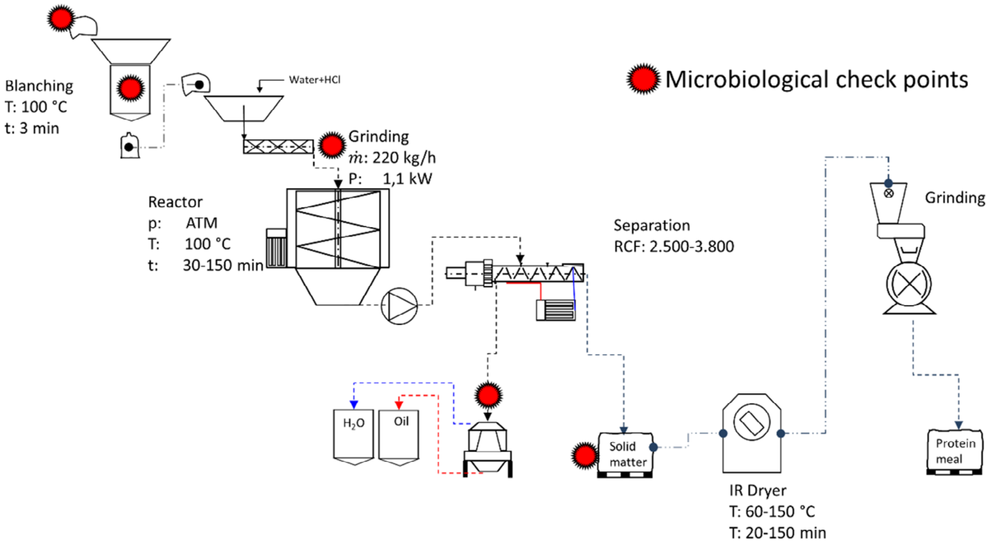

FIGURE 2 | Process diagram of insect wet process.

acid for fat digestion (V_{TEC} = 8 L 0.01 M HCl). This process ran for 60 min after the suspension had reached 60°C, and an end temperature of 100°C was achieved after approximately 15 min. For the technical scale, the digestion was performed in a 97 L heatable stirred tank reactor (Inox Behälter GmbH, EBB90RWAA4-M; heating capacity 12 kW). The separation was done using a two-phase decanter (MD80, LemiTech, Germany; RCF 3800). The mean residence time in the decanter was approximately 70 s. The separation process was operated at 60°C. After separation, the solid phase was dried using an infrared drum dryer (FS-Laborbatch; type FS 60/60–10; Kreyenborg, Germany) at 80°C for 180 min (residual moisture < 14 g/100 g).

2.4 | Sampling Points and Preparation of Samples for Analysis

2.4.1 | Sampling Points

Sampling points included the raw material in all batches. For each batch, at least three samples were taken at each processing stage, except for the drying process, as it was performed on the same raw material for all batches.

In the dry process, additional samples were collected before and after the drying stage, as well as from the press cake and the fat phase. Each drying batch was sampled once. In the wet process, samples were taken from the raw material, blanched larvae, fat melt, protein meal after drying, and the water/fat phase. The sampling points are shown as microbiological checkpoints in Figures 1 and 2.

2.4.2 | Preparation for Analysis

All samples were taken during the steady state of the process. To quantify hygiene parameters, 50 g of the insects, intermediates, and end products were packed in 450 mL sterile bags (SteriBag, Batch J090879; Bürkle, Germany). The samples were stored at a temperature below 5°C until the start of the analysis.

2.5 | Microbiological Analysis

The following analyses were carried out by AniCon Labor GmbH, Emstek, Germany, which operates an accredited laboratory with standardized measurement procedures. As such, each analysis is conducted as an individual assessment, adhering to recognized protocols to ensure accuracy and reliability.

For the total aerobic bacteria count, the method used was DIN EN ISO 4833-2 (2022–05) with a measurement uncertainty of 5.82%. For *Salmonella spp*., the method was DIN EN ISO 6579-1 (2020–08), which was conducted qualitatively; therefore, no measurement uncertainties were calculated. For *Enterobacteriaceae* (*ENT*), the method was DIN EN ISO 21528-2 (2019–05) with a measurement uncertainty of 5.13%. For *E. coli*, the method was DIN ISO 16649-2 (2020–12) with a measurement uncertainty of 3.76%. Lastly, for yeasts and molds, the method used was ASU L 01.00–37 (1991–12) with measurement uncertainties of 6.23% and 8.91%.

The use of standardized methods from an accredited laboratory ensures that the results are consistent and reliable, reflecting the true nature of the samples analyzed.

2.6 | Storage Study for BSFL Protein Feed

The protein meal samples (2.4) from the technical scale wet processes were stored at 15°C for 14 days. The temperature was continuously monitored using [Testo, 608-H1 Hygrometer] to maintain consistent conditions throughout the study. At each storage time point, two samples were prepared and analyzed. Samples were dispatched in duplicate to the laboratory for microbiological analysis (see chapter 2.5) directly after production on day 1, then again after 7 days, and finally after 14 days.

2.7 | Quantification of Furosine

Sample work-up and RP-HPLC were performed based on the protocol of Mayer et al. (2010). Ground insect samples (50 mg) were subjected to acid hydrolysis in 10 mL of 6 M HCl (110°C, 23 h). The samples were filtered, and 200 µL of the filtrate was loaded on RP-18 SPE cartridges previously equilibrated with 5 mL of methanol and 10 mL of double-distilled water. The cartridges were eluted with 3 mL 3 M HCl, the eluate was completely evaporated to dryness using a rotary evaporator, and the residue was taken up in 1 mL of 0.1% TFA. After membrane filtration (0.2 µm), 20 µL was injected into the HPLC Hitachi Elite LaChrom consisting of a pump (L-2130), an autosampler (L-2200), and a diode array detector (L-2455). The separation was performed on a stainless-steel column (250 mm × 4.6 mm, 5 µm) filled with Eurospher C18 material (Knauer, Berlin, Germany) at room temperature at a flow rate of 0.8 mL/min. TFA (0.1%) in water was used as solvent A and a mixture of acetonitrile and water (90/10, v/v) as solvent B. A gradient was formed (0 min, 5% B; 2 min, 5% B; 25 min, 50% B; 27 min, 50% B; 30 min, 5% B; 35 min, 5% B). UV detection was performed at 280 nm. For calibration, we used a furosine standard synthesized according to Henle, Zehetner, and Klostermeyer (1995). Single determinations were carried out on the samples, and two samples of milk (Extended shelf life, ESL) and evaporated milk were included as a standard.

2.8 | Assessment of Hygienization Effectiveness Using *D*-Value in Process Steps

The *D*-value, or decimal reduction time, is a key parameter in food and microbiology for determining the heat resistance of microorganisms. It represents the time required at a constant temperature to reduce the microbial population by 90%. To calculate the *D*-value, the initial microbial count is measured, and the sample is then heated at a fixed temperature for various time intervals. After each interval, the remaining viable count is recorded. Plotting these counts on a semi-logarithmic graph (log of microbial count vs. time) allows for determining the *D*-value as the inverse of the slope. This value is critical for ensuring process safety and control.

For the dry processing method, the *D*-values were calculated for the drying process using data from the raw material and dried insects, as well as for the protein meal derived from the dried insects and the press cake. The number of samples used for these calculations is provided in Table 2. In the wet processing method, the blanching process was assessed by calculating *D*-values from the raw material and blanched insects. Subsequently, the separation and breakdown processes were evaluated using the blanched insects and wet protein meal. The corresponding sample counts for these steps are detailed in Table 3. For the drying step in wet processing, the *D*-value determined during the drying process for dry processing was used, as both drying processes were conducted under identical conditions. The total number of samples used for the *D*-value calculations at the various control points across both processing methods is summarized in Table 6, providing a comprehensive overview.

The *D*-value is calculated using the formula:

$$D = \frac{\Delta t}{\log N_0 - \log N}$$

D = Decimal reduction time [min] Δt=residence time [min]; N_0 = Initial microbial count [CFU/g]; N = Microbial count after Δt [CFU/g].

2.9 | Statistical Analysis

The statistical analysis was performed using Excel (Microsoft, 2013). To determine the difference between process steps, a *t* test analysis (with a significance level of $p = 0.05/0.10$) is done.

3 | Results

The hygiene of animal feed is an important factor for high-performance agriculture, animal welfare, and consumer safety.

TABLE 2 | Hygiene parameters of the dry downstream processing[a].

Control point	Feeding acid[b]	Samples *n*	TBC	*ENT*	*E. coli*	Molds	Yeast	*Salmonella spp.*
Larvae	–	6	8.28	5.48	4.30	4.12	4.04	n.d.
Dry	–	6	5.65**	3.32*	Tr*	3.73	tr	n.d.
Frass	–	3	5.81*	2.26	tr	n.m.	n.m.	n.d.
Protein	–	6	5.56	3.04	tr	2.48	tr	n.d.
Crude oil	–	6	4.08**	tr	tr	tr	tr	n.d.
Larvae	+	4	7.23	n.m.	n.m.	n.m.	n.m.	n.d.
Dry	+	4	3.82	tr	tr	tr	tr	n.d.
Protein	+	4	5.56	tr	tr	tr	tr	n.d.
Crude oil	+	4	2.70	tr	tr	tr	Tr	n.d.

Note: All bacterial counts are presented as colony-forming units per gram (log CFU/g) and *Salmonella spp.* as colony-forming units per 25 g (log CFU/25 g). The reported values represent the mean of the analyses conducted at each sampling point. Not measured (n.m.); limit of quantification log 2.00; traces result below LOQ (tr). *indicates an insignificant reduction in the downstream process as compared to the previous step ($p < 0.10$); **indicates a significant reduction in the downstream process compared to the previous step ($p < 0.05$). Results without * or **indicate a *p*-value higher than 0.10.

[a] Not detectable (n.d.).

[b] Larvae feed pH reduced below 5 with a commercial feeding acids mixture.

In insect processing, the impacts of the processing chain on the microbiological status of the feed have not been investigated until now. This study provides a preliminary assessment of these effects on the hygiene of processed insect protein and fat.

In this study, *Salmonella spp*. were not detected in any sample, and *Clostridium perfringens* was detected only in the protein feed after 14 days of storage. The selected control points for each process (Figure 1 for the dry process and Figure 2 for the wet process) demonstrated varying effects on microbial reduction.

3.1 | Dry Processing and the Influence on the Hygiene Status

3.1.1 | BSFL Farming on a Classical Substrate

The samples intended for hygiene analyses were taken at the control points of the experimental setup. The total bacteria count (TBC, initially 8.28 log CFU/g) decreases during downstream processing (Table 2). In the dry process, the lowest counts were observed in the protein meal (5.56 log CFU/g) and the crude oil phase (4.08 log CFU/g). Over the entire process, a decrease of 2.72 log CFU/g for TBC and 3.22 log CFU/g for *ENT* was observed. Molds showed only a slight decrease during downstream processing, reducing from 4.12 log CFU/g to 2.48 log CFU/g.

3.1.2 | BSFL Farming on a Low-pH Substrate

In this study, *ENT*, *E. coli*, molds and yeast were not detectable when larvae were fed on a substrate with a low pH value (< 5).

3.2 | Wet Processing and the Influence on the Hygiene Status

Samples for hygiene analysis were collected at the control points within the experimental setup. In the technical scale wet process, bacterial counts decreased during downstream processing (Table 3), with the lowest counts observed in the protein meal (4.80 log CFU/g) and crude oil phase (4.04 log CFU/g). Molds and yeasts were only detectable in the raw larvae before processing, and the final protein meal was free of these contaminants. Microbial levels were further monitored in a storage study over 14 days under realistic conditions (Table 4). After 14 days, a slight recontamination was observed in the material, though at levels not considered critical.

3.3 | Estimation of the Maillard Reaction

Within the experiments, the furosine content was determined in selected samples as a parameter to evaluate the Maillard reaction (Table 5). Standards of ESL milk and evaporated milk

TABLE 3 | Hygiene parameters of the wet downstream processing[a].

Control point	Process	Samples *n*	TBC	*ENT*	*E. coli*	Molds	Yeast	*Salmonella spp.*	*C. Perf.*
Larvae	Lab	5	7.77	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Blanching	Lab	5	4.50**	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Cutting	Lab	5	4.84	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Protein	Lab	5	2.23*	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Crude oil	Lab	5	tr*	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Larvae	Tech	4	> 7.00	5.46	4.99	2.00	4.21	n.d.	n.d.
Protein Wet	Tech	4	5.23**	4.15**	3.23**	tr**	tr**	n.d.	n.d.
Protein dry	Tech	4	4.80	tr**	tr**	tr	tr	n.d.	n.d.
Crude oil	Tech	4	4.04**	tr	tr	tr	tr	n.d.	n.d.

Note: All bacteria counts are given in colony-forming units per gram (log CFU/g), *Salmonella ssp.* in colony-forming units per 25 g. 'Lab' refers to a small laboratory scale with 75 g batches, while 'Tech' refers to a technical scale with 25 kg batches. The reported values represent the mean of the analyses conducted at each sampling point. Not detectable (n.d.); Limit of Quantification log 2.00; traces result below LOQ (tr); * indicates an insignificant reduction in the downstream process ($p < 0.10$); ** indicates a significant reduction in the downstream process compared to the previous step ($p < 0.05$). Results without * or ** indicate a p-value higher than 0.10.

[a] Not measured (n.m.).

TABLE 4 | Storage study for BSFL protein feed[a].

Storage time [d]	Samples *n*	TBC	*ENT*	*E. coli*	Molds	Yeast	*Salmonella spp.*	*C. Perf.*
1	2	4.78	tr	tr	tr	tr	n.d.	n.d.
7	2	7.68	2.30	tr	tr	tr	n.d.	n.d.
14	2	4.21	3.87	tr	2.00	2.78	n.d.	2.63

Note: All bacteria counts are given in colony-forming units per gram (log CFU/g), *Salmonella ssp.* in colony-forming units per 25 g. The reported values represent the mean of the analyses conducted at each sampling point.

[a] Not detectable (n.d.), limit of quantification log 2.00, traces result below LOQ (tr).

TABLE 5 | Formation of furosine in infrared drying processes.

Temperature [°C]	Drying time [min]	Moisture [%]	Furosine [mg/kg]
140	0	66.0	65
140	30	60,3	39
140	60	46.0	106
140	90	1,1	1711
130	0	66.2	tr
130	30	60.4	13
130	60	51.9	394
130	120	12.3	301
130	180	0.8	850
70	210	55.1	21
80	210	34.0	73
100	210	13.5	568
120	150	16.0	226–378

Note: Traces result below LOQ (tr).

were included as a quality control. In ESL milk, 38–44 mg/kg furosine was determined (literature data, 7.6–40 mg/kg for indirectly heated ESL milk, assuming a protein content of 3.5%) (Schmidt, Boitz, and Mayer 2014), whereas in evaporated milk, 330–416 mg/kg furosine was determined (literature data, 690 mg/kg for sterilized evaporated milk, assuming a protein content of 7%) (Erbersdobler and Somoza 2007). The formation of furosine during infrared drying processes varied with temperature and drying time. At 140°C, furosine levels increased after 90 min of drying, reaching a peak of 1711 mg/kg, with the initial moisture content decreasing from 66% to 1.1%. At lower temperatures (130°C), furosine levels showed a gradual increase, with the highest measurement at 394 mg/kg after 60 min. Lower temperatures (70°C and 80°C) resulted in minimal furosine formation, while drying at 100°C and 120°C produced intermediate levels of 568 mg/kg and 226–378 mg/kg, respectively.

3.4 | *D*-Value of Critical Processing Step

The calculated *D*-values are presented in Table 6. A clear trend is observed, indicating that *Enterobacteriaceae* exhibit higher heat tolerance compared to total microbial counts. The *D*-values vary significantly between different processing steps, with examples such as the drying process showing *D*-values of 91 and 111 min, and the defatting step displaying *D*-values of 3 and 18 min, respectively.

4 | Discussion

In this study, the general microbiological status was monitored at different stages of insect processing, from raw BSFL to protein meal and crude oil. The results reveal significant differences between dry and wet processing methods. The objective of this study is to identify critical processing steps concerning feed safety and the microbiological status of BSFL products, providing a preliminary framework for manufacturing guidelines. Regulation (EU) No. 142/2011 (Table 1) is set as a critical marker for a commercial end product. Both processes start with similar microbial quantities (TBC 8.28–7.23 log CFU/g, *Enterobacteriaceae* 5.48–5.46 log CFU/g, *Escherichia coli* 4.99–4.30 log CFU/g, molds 4.12–2.00 log CFU/g, and yeast 4.21–4.04 log CFU/g). *Salmonella ssp.* and *Clostridium perfringens* were not detectable in any sample. Contamination with *Salmonella ssp.* or *Clostridium perfringens* occurs through the feeding substrate during rearing (Vandeweyer et al. 2021; Van Looveren et al. 2022). Under the chosen conditions, both processes have shown a highly different effect on microorganisms. Figure 3 displays the course of the TBC for both methods within the previously defined critical process steps. The *D*-values for the thermal processing steps, calculated from the data, are presented in Table 6. The individual procedures are discussed below.

TABLE 6 | *D*-value of dry and wet downstream processing.

Process	Samples *n*	D-value$_{\mathrm{TBC}}$	D-value$_{ENT}$
Dry processing			
Drying	12	91	111
Defatting	10	3	18
Wet processing—Lab scale			
Blanching	10	1	—
Fat digestion and separation	10	29	—
Wet processing—Lab scale			
Fat digestion and separation	4	68	92
Drying	4	91	111

Note: All *D*-Value are presented as minutes (min). The *D*-values are calculated from the mean of the analyses conducted at each sampling point.

4.1 | Dry Processing

Most studies on microbial safety focus on raw and dried insects, rather than on the processed animal protein used in the feed industry (Grabowski and Klein 2017). After drying, a significant reduction in general microorganisms (TBC) between 2.63 and 3.41 log CFU/g ($p > 0.05$) was observed. The critical parameter *ENT* showed a reduction of 2.16 log CFU/g ($p > 0.10$) during the drying step. It was also demonstrated that the majority of microorganisms reduced in the dryer belong to the *Enterobacteriaceae* family. Specifically, the quantity of *E. coli* experienced a strong reduction by 4.30 log CFU/g, making it undetectable in the product after this processing step. Under the specified drying conditions (T: 90°C; t: 240 min; DM: <94%), *D*-values of 91 min for TBC and 111 min for *ENT* were determined, indicating the necessary treatment time at

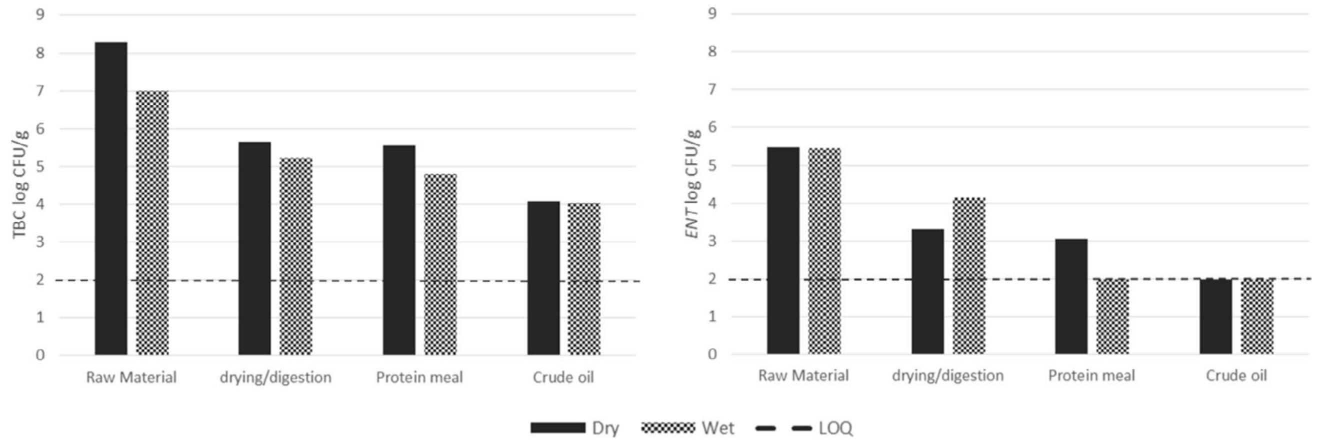

FIGURE 3 | Influence of different process steps of the dry and wet process on the TBC of intermediate and end products.

these conditions to achieve a one-log cycle reduction in the bacterial population. Therefore, the chosen infrared drier technology in this study is unsuitable for drying insects from approximately 70 g/100 g residual moisture to below 6 g/100 g, requiring an extended residence time for the drying step. Infrared drying demonstrates insufficient evaporation capacity for this product type; hence, industrial-scale drying will utilize microwave technology. Despite a residence time of 240 min and energy input, the observed *D*-values (TBC: 91; *ENT*: 111 min) remain notably high, suggesting that most microorganisms are located inside the larvae's gut rather than on the surface where infrared heating is effective. To optimize the *D*-value in this process step, adjusting the dryer's temperature or residence time is feasible. The *D*-value is sensitive to process condition changes; for instance, increasing the IR-drying temperature significantly reduces the *D*-value but accelerates the Maillard reaction, generating more roasting products. Data from Table 5 indicate that drying temperature significantly influences product quality, with the furosine content used to assess the Maillard reaction, particularly increasing with higher dry matter content during drying. The most substantial increase occurs in the final drying segment, necessitating dehydration in the dry process to achieve approximately 4% residual moisture. Screw presses cannot operate at higher moisture levels, which would prevent pressing. The products of this reaction have a negative influence on the nutritional value of the end product. Generally, the drying process has a positive effect on product hygiene, but the reduction potential (*D*-value 91/111 min) is insufficient for generating a commercial end product due to high *ENT* quantities (3.32 log CFU/g). Furthermore, the choice of drying technology has a significant impact on achieving a low *D*-value. For example, switching from infrared to microwave drying alters the heating mechanism, potentially intensifying the impact of microwaves on microorganisms inside the larvae's gut compared to infrared heating. On an industrial scale where microwave drying is prevalent, the dry process does not exhibit these acute microorganism-related issues. Additionally, Van Looveren et al. (2022) note that various drying techniques inadequately reduce the bacterial count in dry insects highly contaminated with *C. perfringens*. Spore-forming bacteria present a distinct challenge for process technology, although detailed discussion is beyond the scope of this study.

Nyangena et al. (2020) investigated drying methods applied in East Africa and their potential for microbial reduction. It was found that the processing methods used (boiling, toasting, and drying) have different effects on the microbiota. Similar to this study, it was demonstrated that boiling is an effective measure to reduce microbial counts, while drying in an oven and sun drying did not yield strong reduction rates. Generally, the drying data are comparable to the findings of this study. Furthermore, the data pool can now be expanded to include further processing, especially regarding de-oiling.

The EU Regulation 142/2009 sets strict requirements for the hygiene and processing of animal by-products, which are also relevant for insect processing. In particular, the regulation demands a significant reduction of pathogenic microorganisms to ensure food safety. In this study, the *D*-values (TBC: 91; *ENT*: 111 min) indicate inadequate reduction, which does not meet the regulation's requirements for nearly complete elimination of pathogenic germs. This is also confirmed by the high quantity of *ENT* (3.32 log CFU/g), which exceeds the limits set by the regulation. Furthermore, the regulation emphasizes the necessity of process validations to ensure that the employed procedures (e.g., drying, de-oiling) meet the required hygiene standards. This study found that the infrared drying technology used is insufficient to achieve the required hygiene standards. A switch to microwave technology could provide a solution that reduces the *D*-value and ensures better microbial reduction, thus better aligning with the requirements of EU Regulation 142/2009.

De-oiling by a screw press is a common technique in insect dry processing. The residence time in the de-oiling section is approx. 300 s at a temperature between 70°C and 80°C. The *D*-value at the de-oiling process is 3 min for TBC and 18 min for *ENT*. In this study, there was no additional reduction effect of the microorganism quantity observed in the products by this step. Considering the *D*-value at the de-oiling conditions, the residence time in the machine, which was not varied in this study, does not allow a stronger reduction of bacteria counts in the products. This is the reason for applying the low-temperature pressing technique, which was used in this study. Also to decrease the *D*-value, the machinery configuration must be changed. The preconditioning of the dry insects before the screw press was realised at a temperature of 45°C by indirect heat. Due to this preconditioning step, the residence time in the screw press is reduced to only a few seconds at a temperature below 100°C. In this study, it was not possible to generate a commercially safe end product without an extra hygienization step (Table 2). For a technical scale-up, it is important to consider that the residence time, temperatures, and system pressure of an industrial system are much higher than those of a small laboratory system. Thus, the *D*-values are not directly transferable but provide a starting point for the assessment of critical control points. To reduce the amount of microbial contamination of the raw material, it is possible to use acids (formic acid, acetic acid, or propionic acid) as feed additives in insect farming, as is the case with insects from the company FarmInsects (Germany) that were also used in this study. Here, *ENT* and *E. coli* are not detectable during the whole process (Table 2). With this raw material, it is possible to generate a commercially safe product without an extra hygienization step (Table 2).

4.2 | Wet Processing

The first part of the results was generated in small-scale experiments. These results were used to define the control points in the large-scale (50 L-batch) experiments. The small-scale experiments have shown two influential process steps with a high hygienization potential. The blanching reduces the TBC by approximately 3.27 log CFU/g ($p > 0.05$). After blanching the BSFL to hygienize them, they are cut to inactivate endogenous enzymes (phenol oxidase, protease, etc.; blanching inhibits enzymatic browning processes and protein degradation). This handling step of the sensitive product does not show any effect on the microbial quantity. The blanching process has shown a *D*-value of 57 s for TBC. Acid digestion to produce better fat yields has also shown a significant influence on TBC, with significant reduction rates of 2.61 ($p > 0.10$) log cycles and more observed. After digestion and fat separation by centrifugation, the TBC in the liquid phase (crude oil) is below 2.00, and in the protein meal, it is at 2.23 log CFU/g. For fat digestion and extraction, a *D*-value of 29 min for TBC was determined. This experiment resulted in the possibility of describing the control points on a technical scale. These include the raw product, the blanched BSFL, process intermediates after fat separation, and the dry product.

For wet processing, only a few hygiene data have been published, but the regulations in the area of carcass utilization can be considered safe. Therefore, the result that wet processing does not pose a direct risk to food and feed safety and that the legal framework conditions can be met was not surprising.

In the large-scale experiment, BSFL were used that were devitalized by freezing. Assuming that fat digestion has a sufficiently high hygienization potential under the process conditions, it is possible to omit the blanching step in the process scheme. Under the selected process conditions (digestion: t: 60 min; T: 60°C; separation: residence time: 70 s, T: 60°C), it was possible to significantly reduce the TBC by 1.77 and the *Enterobacteriaceae* by 1.31 log CFU/g ($p > 0.05$). The reduction of *Escherichia coli* by 1.76 log CFU/g indicates that a significant portion of the bacterial reduction in this process step is attributable to *Escherichia coli*. For the complete process (digestion and separation), a *D*-value of 68 min for TBC and 92 min for *ENT* was determined. The moist protein meal was dried by infrared radiation (t: 240 min; T: 80°C) to a residual moisture below 14 g/100 g. No *ENT*, yeasts, or molds were detectable in the dried end product. Drying of the intermediate product under conditions similar to the dry process shows lower *D*-values for TBC (91 min) and ENT (below 111 min). The bacterial contamination of the dried product was below the requirements of regulation (EU) 142/2011.

4.3 | Storage Study

The storage study of wet-processed protein meal at 15°C for 14 days demonstrated a gradual recontamination of the feed material. Within the first 7 days, an increase in the TBC of 2.90 log CFU/g was observed. The *ENT* increased from below the limit of detection to 2.30 log CFU/g. After 14 days of storage at 15°C, an additional increase in *Enterobacteriaceae* to 3.87 log CFU/g was noted in the protein meal. Furthermore, this study detected *Clostridium perfringens* for the first time in an insect product (2.63 log CFU/g). According to the recommendations of Regulation (EU) No. 142/2011, the feed material would be considered microbiologically unstable after 14 days of storage and would be prohibited for use as feed in the European Union. For longer storage periods, a higher decontamination potential is required.

5 | Conclusions

In general, insects are a very attractive alternative in the field of animal nutrition. By employing various processing methods, a variety of end products can be produced. The present study is a preliminary attempt to compare two different processes, revealing significant differences between the wet and dry processing methods. This study highlights critical process points along the downstream process steps. These critical points include the raw material, the process steps of devitalization, drying or digestion, as well as separation and storage conditions. Scaling up technical processes from a small laboratory system to an industrial system presents a major challenge. It is important to consider all process parameters (residence time, temperature profiles, system pressure, etc.) individually in this scaling procedure. Consequently, the *D*-values are not directly transferable but provide a starting point for assessing critical control points. In this study, a first trend indicates that dry heat treatment shows a lower hygienization potential than wet heat treatment in insect processing. To scale-up these results, each process diagram needs to be analyzed separately. In the dry process, without additional technical hygienization, for example, by blanching, it is only possible to reliably comply with the legal requirements by continuously monitoring the raw materials. Due to the dry heat within the drying process used in this study (IR-drying), only a small reduction in the number of microbes can be achieved. From a feed legislation perspective, a hydrothermal process step is therefore required for hygienization. In contrast, the wet process demonstrates a very high bacterial count reduction within the standard process steps. The notably higher hygienization effect of the wet process compared to the dry process is due to the moist heat during blanching and cell disruption. In this scenario, heat transfer to the microorganisms is much more effective, ensuring a reliable and high devitalization rate. Thus, more heavily contaminated raw materials can also be processed using the wet process. The selection of a suitable processing method depends on several factors, such as the desired end product quality, throughput rate, the nature of the raw materials, and storage logistics. To our knowledge, the effects of lipid oxidation during processing are not yet sufficiently clarified. It is also possible that processing negatively affects the lipid quality, which is also a potential raw material for feed production. Future studies should investigate the microbiota inside the larvae along the process and examine individual chemical parameters of browning and lipid oxidation in greater detail.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Belluco, S., C. Losasso, M. Maggioletti, C. C. Alonzi, M. G. Paoletti, and A. Ricci. 2013. "Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review." *Comprehensive Reviews in Food Science and Food Safety* 12: 296–313.

Čičková, H., G. L. Newton, R. C. Lacy, and M. Kozánek. 2015. "The Use of Fly Larvae for Organic Waste Treatment." *Waste Management* 35: 68–80.

Erbersdobler, H., and V. Somoza. 2007. "Forty Years of Furosine—Forty Years of Using Maillard Reaction Products as Indicators of the Nutritional Quality of Foods." *Molecular Nutrition & Food Research* 56: 104–109.

European Food Safety Authority. 2015. "Scientific Opinion on the Safety of the Use of Dimethyl Ether as an Extraction Solvent Under the Intended Conditions of Use and the Proposed Maximum Residual Limits." *EFSA Journal* 13: 10.

Federal Ministry of Education and Research (BMBF). 2020. "Nationale Bioökonomiestrategie für eine nachhaltige, kreislauforientierte und starke Wirtschaft (in German). Press release 003/2020."

Food and Agriculture Organization of the United Nations. 2013. "Edible Insects—Future Prospects for Food and Feed Security."

Food and Agriculture Organization of the United Nations (FAO). 2011. "Global Food Losses and Food Waste, Rome." ISBN 978-92-5-107205-9.

Grabowski, N. T., and G. Klein. 2017. "Microbiological Analysis of Raw Edible Insects." *Journal of Insects as Food and Feed* 3: 7–14.

Henle, T., G. Zehetner, and H. Klostermeyer. 1995. "Fast and Sensitive Determination of Furosine." *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* 200: 235–237.

Makka, H. P. S., G. Tran, V. Heuzé, and P. Ankers. 2014. "State-Of-The-Art on Use of Insects as Animal Feed." *Animal Feed Science and Technology* 197: 1–33.

Mayer, H. K., B. Raba, J. Meier, and A. Schmid. 2010. "RP-HPLC Analysis of Furosine and Acid-Soluble β-Lactoglobulin to Assess the Heat Load of Extended Shelf Life Milk Samples in Austria." *Dairy Science & Technology* 90: 413–428.

Nyangena, D. N., C. Mutungi, S. Imathiu, et al. 2020. "Effects of Traditional Processing Techniques on the Nutritional and Microbiological Quality of Four Edible Insect Species Used for Food and Feed in East Africa." *Food* 9: 574.

Oonincx, D. G. A. B., S. Van Broekhoven, A. Van Huis, and J. J. A. Van Loon. 2015. "Feed Conversion, Survival and Development, and Composition of Four Insect Species on Diets Composed of Food By-Products." *PLoS ONE* 10: e0222043.

Schmidt, A., L. I. Boitz, and K. Mayer. 2014. "A New UHPLC Method for the Quantitation of Furosine as Heat Load Indicator in Commercial Liquid Milk." *Journal of Food Composition and Analysis* 56: 104–109.

Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC). 2014. "Food Safety Aspects of Insects Intended for Human Consumption. Sci Com 2014/04 and SHC Nr. 9160."

Sindermann, D., J. Heidhues, S. Kirchner, N. Stadermann, and A. Kühl. 2021. "Industrial Processing Technologies for Insect Larvae." *Journal of Insects as Food and Feed* 7: 857–875.

Sudwischer, P., and W. Sitzmann. 2022. "Futtermittelrechtliche Eckpfeiler—Was gilt für Insekten als Eiweißfuttermittel?" *Mühle + Mischfutter* In German 21: 18–19.

Van Looveren, N., D. Vandeweyer, J. van Schelt, and L. Van Campenhout. 2022. "Occurrence of ***Clostridium perfringens*** Vegetative Cells and Spores Throughout an Industrial Production Process of Black Soldier Fly Larvae (***Hermetia illucens***)." *Journal of Insects as Food and Feed* 8: 399–407.

Van Rooijen, C., G. Bosch, A. F. B. van der Poel, P. A. Wierenga, L. Akexander, and W. H. Hendriks. 2013. "The Maillard Reaction and Pet Food Processing: Effects on Nutritive Value and Pet Health." *Nutrition Research Reviews* 26, no. 2: 130–148.

Vandeweyer, D., J. De Smet, N. Van Looveren, and L. Van Campenhout. 2021. "Biological Contaminants in Insects as Food and Feed." *Journal of Insects as Food and Feed* 7: 807–822.