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Comparison of black soldier fly larvae pre-treatments and drying techniques on the microbial load and physico-chemical characteristics

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Abstract

Black soldier fly larvae (BSFL) are good candidates for upcycling wet organic residuals. Like other unprocessed raw animal products, BSFL require processing to prevent spoilage and degradation during storage and to facilitate their use as feed ingredients. In this study, hot-air drying and freeze-drying were examined as means to ensure long-term preservation. Pre-treatments of larvae, such as puncturing, blanching (40 s) and scalding (2, 4, 6 and 8 min) in boiling water reduced drying times, most likely by affecting the integrity of the wax-coated cuticle that protects the larvae against desiccation. Overall, the larvae dried two to six times faster using hot-air compared to freeze-drying, and larvae pre-treatments were proven to effectively improve drying efficacy. Pre-treating larvae in boiling water followed by a shorter drying time with hot air was effective at reducing primary and secondary oxidation as well as darkening/browning (colour lightness, L* value) compared to the untreated control (raw-thawed) larvae. The larvae pre-treatments in boiling water also led to a significant reduction in microbial load (3.21 to 4.83 log) in the dry product compared to the control. BSFL powder, produced from grinding thawed larvae that were pre-treated for 4 min in boiling water before being dried in hot air (60 °C; 6 h), had a water activity below 0.4. This led to a relatively stable product with limited colour changes over a 30-day storage period. These processing treatments also resulted in a product with no detectable *Salmonella* and *Escherichia coli* counts ranging from 100 to 1000 cfu/g. Overall, the powdered BSFL product was deemed suitable to incorporate into pelleted feed under the current regulations in Canada.

Keywords: blanching, freeze-drying, hot-air drying, insect, scalding

1. Introduction

Over the last decade, the concept of insect farming as a sustainable source of food and feed has been extensively reviewed (Baiano, 2020; Rumpold and Schlüter, 2013a,b; Sun-Waterhouse *et al.*, 2016; Valeras, 2019; Van Huis, 2013). The advantage of raising edible insects lies in the ability to produce quality nutrients with high feed conversion efficiency, over short production periods and with a limited footprint. The potential for some insects to be produced on residual organic materials is certainly one of the greatest advantages, as it provides new avenues for waste management (Berggren *et al.*, 2019; Gold *et al.*, 2018; Zurbrügg *et al.*, 2018).

The ability of black soldier fly larvae (BSFL; *Hermetia illucens*) to upcycle various waste streams has been widely demonstrated (review by Gold *et al.*, 2018; Lalander *et al.*, 2019). Given their saprophagous nature, BSFL can be effectively produced on poultry feed, fruit and vegetable waste, milling and brewery side streams, abattoir waste, municipal organic solid waste, human and animal manure, and sludge with relatively short development times (larval stage of 15 to 50 days). At harvest, BSFL contain good quality fatty acids, proteins, and micronutrients at levels that may vary according to the rearing conditions and feeding substrates (Barragán-Fonseca, 2018; Cammack and Tomberlin, 2017; Lalander *et al.*, 2019; Nguyen *et al.*, 2013).

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In Canada, BSFL are primarily produced on pre-consumer fruit and vegetable residues to reduce feed safety risks associated with pathogen growth (Cappellozza *et al.*, 2019). Each insect product must undergo a thorough evaluation of the safety and/or efficacy of its use in the diet of the intended livestock (CFIA, 2019). This includes an evaluation of the physical, chemical and biological risks to the insect itself, the manufacturing process and the final feed ingredients. To date, only whole, dried larvae have received accreditation as a novel feed for fish and poultry in Canada (Minister of Justice Canada, 2021). However, larvae in a powdered form would ease the incorporation into feed.

Considering that organic residue may already contain high microbial loads, that larvae produce their excreta in the environment in which they grow and that they are processed as whole larvae with intact intestinal tracts, the risks of encountering microbiological pathogens in the final product are high (Bruno *et al.*, 2019; De Smet *et al.,* 2018; Gold *et al.*, 2020; Lalander *et al.*, 2019; Shelomi *et al.*, 2020; Tanga *et al.*, 2021; Wynants *et al.*, 2019). Food waste should be stabilised to reduce these risks upstream of production. Moreover, a period of feed withdrawal to allow the gastro-intestinal tract to be purged could be considered, as is done for large farm animals before slaughter. However, at least one study has demonstrated no improvement in larval microbial load as a result of this process (Larouche, 2019) and can alter gut microbiome (Yang *et al.,* 2021). In all cases, the nutrient composition, near-neutral pH (7.5±0.2; Larouche *et al.*, 2019) and high microbial load contribute to the perishable nature of raw insects and further processing is necessary to stabilise products (Grabowski and Klein, 2017; Melgar-Lalanne *et al.*, 2019; Sun-Waterhouse *et al.*, 2016; Zhen *et al.*, 2020). Processing techniques must be cost effective for producers and significantly reduce microbiological risks during prolonged storage, all while maintaining nutrient integrity and quality.

Drying (such as roasting, frying, sun-drying, freeze-drying and microwave-assisted drying) is a commonly used technique for increasing the shelf-life of edible insects (Melgar-Lalanne *et al.*, 2019). Oven-drying and freeze-drying are the preferred methods for dehydrating whole edible insects (Melgar-Lalanne *et al.*, 2019). In general, oven (convective) drying is most commonly used since it is the least expensive among the more efficient drying processes, and in addition, it can be easily adapted to continuous industrial operations. Conversely, when the quality of the dried product is prioritised, freeze-drying is most often used, despite it being costly and requiring batch processing (Ratti, 2001). Drying reduces the water activity (a_w) in feedstuff, which protects against degradative reactions, such as enzymatic reactions, and spoilage from microbiological activity during storage. No microorganisms are able to grow at an a_w below 0.6. During the drying process, cells are subjected to osmotic pressure and are exposed to reactive oxygen species that damage macromolecules and membranes as water evaporates (Lang *et al.,* 2016). Despite cell injury, dried goods are not sterile and surviving pathogens are able to grow upon rehydration (Lang *et al.*, 2016). Therefore, blanching prior to drying is a common practice for most commercialised edible insects in order to reduce initial microbial counts and to inactivate enzymes, but would not be severe enough to inactivate bacterial spores (Kamau *et al.*, 2018; Jay, 1992; Larouche *et al.*, 2019; Melgar-Lalanne *et al.*, 2019; Vandeweyer *et al.*, 2017; Zhen *et al.*, 2020).

Hot-air drying results in dehydrated products that can have an extended shelf life of up to a year when stored at room temperature. Unfortunately, these processes often drastically impact the quality of the product affecting attributes such as colour, and leading to component denaturation, compound oxidation, vitamin deterioration, etc. (Larouche *et al.*, 2019; Ratti, 2001). Vacuum freeze-drying is the best method for water removal and results in products with the highest quality (due to the absence of oxygen and the low temperatures used during the process), but this dehydration method is expensive (Ratti, 2001). There are a number of recent studies on the drying of insects (mealworm larvae, crickets, BSF) using hot-air drying, microwave drying, freeze-drying and/or fluidised-bed drying, and the impact on the properties of the product (protein quality and functionality, amino acid composition, *in vitro* digestibility; Huang *et al.,* 2019; Purschke *et al.,* 2018; Vandeweyer *et al.,* 2017; Viera-Alvez *et al.,* 2016). However, to our knowledge, the impact of drying methods on final lipid quality and oxidation has not been previously reported. Understanding the impact on lipid oxidation is important, as it can reduce the nutritional value of the product, negatively affect sensory attributes (Purriños *et al.,* 2011) and produce harmful compounds (Yi *et al.,* 2013) detrimental to the animals that consume the feed/product.

This study reports the impact of larvae pre-treatments (puncture or in boiling water) and drying processes (hot-air drying and freeze-drying) on the final BSFL products. Larvae pre-treatments were performed prior to hot-air drying and freeze-drying in order to reduce microbial load, ease water evaporation and ultimately to reduce water activity in the final product to a level that will allow long-term storage while minimising any loss in nutritional value. Impacts on the drying rate, colour and lipid oxidation are also evaluated. The selected processing treatments are expected to effectively reduce the microbial load and contamination levels in BSFL that are intended for consumption as animal feed.

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2. Material and methods

Larvae production

The BSFL used during these experiments were sourced from our colony at the Laboratoire de recherche en sciences aquatiques (LARSA) at Université Laval (Quebec City, QC, Canada). The larvae were produced in 5-l plastic buckets that were incubated in the dark at a constant temperature (27 °C) and relative humidity (80% RH; MLR-350, Sanyo, Osaka, Japan). For the first four days post-hatching, larvae were fed *ad libitum* on a diet (50% wheat bran, 20% corn meal, 30% alfalfa meal with water added until a final moisture content of 70% was reached; Hogsette, 1992) that contained a fungicide (0.15% methyl 4-hydroxybenzoate; Sigma-Aldrich, Oakville, ON, Canada; Bruno *et al.*, 2019) to control mould development. On day four, larvae were sieved to adjust density and fed for six days on the Gainesville diet without fungicide at a feeding rate of 100 mg/larvae/day. On day 10, larvae (17.0±2.2 mm length, 5.2±0.3 mm width, 0.14±0.03 g weight) were harvested by immersing in water, sieved and rinsed under running water, followed by rinsing with distilled water. They were then patted dry with absorbent paper and vacuum packaged (Eco Vacuum Pro, Orved S.P.A, Venice, Italy) in polyamide/polyethylene (20/80) channelled vacuum bags (CB100; Orved S.P.A.; total thickness of 100 um/3.6 mil., O_2 transmission rate at 65 cm^3/m^2 × 24 h at 23 °C/0% r.F./RH, water vapour transmission rate at 2.5 g/m^2 × 24 h at 23 °C/85% r.F./RH). Larvae were then euthanised by freezing and stored at -40 °C. Hence, raw-thawed larvae were the initial material used for pre-treatments and drying. Fresh larvae were kept on ice until they were vacuum packaged.

Pre-treatment of larvae before drying

Whole larvae were thawed overnight at 4 °C in the dark. To facilitate water evaporation through the larval cuticle different pre-treatments were applied. Larvae were punctured (50 µm holes) using a sterile mechanical boring apparatus (Figure S1). Larvae (30-g batches in triplicate) were blanched for 40 s, or scalded for 2, 4, 6 or 8 min in boiling water using a 5-l vessel equipped with a stainless-steel perforated sieving basket to remove the larvae instantly. Temperature was monitored to ensure constant water temperature during treatment.

Drying conditions

Frozen larvae (-40 °C) were hot-air dried (Model UOP8-G, Armfield, Hamsphire, UK) at 60 °C, at an air speed of 1.5 m/s and an RH of 30% for up to 14 h. Freeze-drying (Freeze mobile 25 l Vir Tis Company, Gardiner, NY, USA) was performed at a constant shelve temperature (40 °C) under a 0.03 Torr vacuum for up to 72 h. To determine optimal drying conditions, drying curves were determined as described by Caparros Megido *et al.* (2017). Briefly, drying kinetics were followed by plotting relative water content, X/X_0, over drying time. Dry mass (D*m*) was determined using a vacuum oven (Isotemp Vacuum Oven Model 281A, Fisher Scientific, Waltham, MA, USA) in the presence of a desiccant (P_2O_5) at 60 °C and 25 Torr for 48 h (Araya-Farias *et al.*, 2011). Samples were then cooled to room temperature in a desiccator and precisely weighed (±0.001 g). The X/X_0 ratio was calculated as follows:

$$\frac{X}{X_o} = \frac{W_0 - Dm}{W_f - Dm} \quad (1)$$

where X is the water content of the product after hot-air drying or freeze-drying based on dry basis (g of water/g of dry mass); X_0, water content on dry basis of the initial product; W_0 is the weight of the initial product (g), W_f is the weight of the product after hot-air drying or freeze-drying (g), and D*m* is the dry mass, or the final weight of the product after completely dried in a vacuum oven (g).

Quality indicators

Proximal analyses, lipid oxidation levels and physico-chemical characteristics were evaluated for the ground dry larvae and after pre-treatment of larvae and drying by hot-air or freeze-drying. After freeze-drying or hot-air drying, larvae were stored overnight in desiccators containing $CaSO_4$ before being ground (Magic Bullet, Model MB1001, Taylorsville, KY, USA), and stored at -20 °C until further analysis.

Proximal analysis

Contents of dry matter (DM) and ash were evaluated in triplicate using procedures AOAC 934.01 and AOAC 942.05, respectively (AOAC, 2016). To determine DM content, DM was first obtained by drying the sample in a vacuum oven at 98 °C (Vacuum Oven LabLine, model 6273, Thermo Fisher Scientific, Marietta, OH, USA). Ash content was determined by incineration of the DM (Lindberg/Blue M Box Furnace, model BF51828C-1, Thermo Fisher Scientific, Ashville, NC, USA) at 600 °C for 13 h.

Total nitrogen content was analysed using the LECO method (Truspec model FP528, Leco Corp., St. Joseph, MO, USA) according to procedure AOAC 992.15 (AOAC, 2016). The protein content was calculated using the nitrogen-to-protein conversion factor Kp=4.76, determined by Janssen *et al.* (2017). Protein content is expressed as a percentage (%) of the dry matter and analyses were performed in triplicate.

To evaluate total lipids, an ether extraction on freeze-dried ground larvae was performed according to method AOCS AM5-04 from the American Oil Chemists' Society (1998). Extraction was performed with ethyl ether (120 min

Table 1. Physico-chemical properties of black soldier fly larvae after pre-treatment in boiling water.[1]

Parameters[2]	Raw thawed[3]	Blanching	Scalding			
		40 s	2 min	4 min	6 min	8 min
pH	6.5±0.1^{d}	8.6±0.1^{b}	8.9±0.1^{a}	8.8±0.2^{a}	8.5±0.1^{c}	8.5±0.1^{c}
DM (%)	22.1±0.1^{a}	21.5±0.4^{a}	21.0±0.3^{ab}	20.0±0.2^{bc}	19.8±0.1^{c}	18.6±0.7^{d}
Ash (%)	10.3±0.1	10.2±0.1	10.3±0.3	10.4±0.3	9.9±0.7	10.4±0.3
XO (nmol eq. CHP/g)	198.4±30.3	152.0±5.8	210.4±39.6	175.0±36.2	158.2±53.7	148.6±15.8
TBARS (nmol MDA/g)	58.9±4.1^{a}	46.0±3.7^{b}	53.3±1.5^{ab}	49.7±4.9^{b}	46.1±0.4^{b}	44.5±2.5^{b}
L*	49.2±0.1^{a}	37.5±0.4^{b}	31.4±1.2^{d}	31.5±0.1^{d}	38.4±0.1^{b}	33.8±0.2^{c}
a*	3.7±0.1^{a}	2.8±0.1^{b}	1.0±0.2^{d}	1.7±0.1^{c}	1.4±0.1^{c}	1.4±0.1^{c}
b*	20.1±0.2^{a}	13.5±0.1^{b}	8.00±0.3^{c}	7.1±0.1^{d}	8.5±0.1^{b}	8.3±0.1^{bc}
ΔE[4]	Reference	13.1±0.1^{d}	21.6±1.1^{a}	21.9±0.1^{a}	15.9±0.1^{c}	19.4±0.2^{b}
C*	20.2±0.1^{a}	13.8±0.1^{b}	8.0±0.3^{d}	7.3±0.1^{e}	8.6±0.1^{c}	8.4±0.1^{c}
h*	79.7±0.1^{bc}	78.4±0.6^{c}	82.6±1.5^{a}	76.6±0.1^{c}	80.7±0.1^{b}	80.1±0.1^{bc}

[1] Data represent means ± standard deviations (n=6). NS = not significant. Values with different letters in the same row are significantly different ($P<0.05$).

[2] DM = dry matter; CHP = cumene hydroperoxide; eq. = equivalent; MDA = malonaldehyde; TBARS = thiobarbituric acid reactive substances; XO = xylenol orange; results are expressed as a percentage (%) of the dry matter.

[3] Live larvae euthanised by freezing at -40 °C and then thawed prior to drying.

[4] $\Delta E = [(L^*_{ref.}-L^*_{treated})^2 + (a^*_{ref}-a^*_{treated})^2 + (b^*_{ref}-b^*_{treated})^2]^{1/2}$ indicates the colour difference using thawed raw-larvae as the reference. Reference larvae were assigned a value of zero. Chroma value $C^* = (a^{*2}+b^{*2})^{1/2}$ measures colour intensity. Hue angle $h = Tan^{-1}(b^*/a^*)$ measures colour appearance.

at 90 °C) on hydrolysed samples (4 N hydrochloric acid, 60 min, 90 °C) using TX4 filters (XT15 Extractor, ANKOM Technology, New York, NY, USA). Lipid content is expressed as a percentage (%) of the dry matter and analyses were performed in triplicate.

Lipid oxidation

Products from primary (hydroperoxides) and secondary oxidation were quantified using a modified version of the Ferrous Oxidation-Xylenol Orange (FOX; Grau *et al.*, 2000; Hermes-Lima *et al.*, 1995) method and the thiobarbituric acid reactive substances (TBARS) method (Joanisse and Storey, 1998; Uchiyama and Mihara, 1978), respectively. The measurements were collected for 3.5 g of shredded raw-thawed larvae or 1 g of dry ground larvae. All analyses were performed in triplicate.

For the FOX analyses, samples were homogenised (VDI 25, VWR, Radnor, PA, USA) with 100% HPLC-grade cold methanol (1:5, m:v) for 45 s for shredded raw-thawed larvae and 1 min for dry ground larvae. Homogenates were then centrifuged at 3,000×*g*, for 10 min at 4 °C. Next, the supernatant was centrifuged for a second time (3,000×*g*, 5 min at 4 °C) and kept on ice in the dark. The reagents were sequentially added and mixed (250 µl of a 1 mM aqueous solution of $(NH_4)_2Fe(SO_4)_2$, 100 µl of 25 mM H_2SO_4 in methanol, 100 µl of 0.1 mM xylenol orange in methanol, 450 µl of methanol and 100 µl of the supernatant), and a blank solution was prepared using methanol. Reaction mixtures were incubated in the dark at room temperature (21 °C) for 1 h. Next, the absorbance was measured at 580 nm against the blank (Varioskan™, Thermo Electron Corporation, Vantaa, Finland). The quantity of hydroperoxides was evaluated using a standard curve generated from a solution of known concentrations of cumene hydroperoxide (CHP) in methanol. Results are expressed as nmoles of CHP equivalents per g of dry larvae.

TBARS results are expressed as malondialdehyde (MDA) equivalents (Joanisse and Storey, 1998). Samples were first homogenised in 10 ml of 1.15% phosphoric acid, centrifuged two times and kept in the dark, as described for the FOX analyses. In glass tubes, 400 µl of the supernatant was mixed with 400 µl of thiobarbituric acid solution (TBA; 1% thiobarbiturique acid and 0.1 mM butylated hydroxytoluene in 0.05 M NaOH). For the blank solution, 400 µl of 3 mM HCl was used to replace the TBA solution. The standard solution consisted of 400 µl of the known concentration of MDA and 400 µl of the TBA solution. All the tubes were treated with 200 µl of 7% phosphoric acid before being incubated at 100 °C for 15 min. After cooling to room temperature in the dark for 10 min, the TBA-aldehyde complex was extracted with 1.5 ml of n-butanol, mixed for 1 min and centrifuged (2,000×*g*, 5 min at 4 °C) to separate the organic phase from the aqueous phase. Absorbances of the organic phase were measured at 532 nm and 600 nm using a Varioskan spectrophotometer. Changes

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in absorbance (A_{MDA}=A_{532}-A_{600}) were used to calculate MDA concentrations. The quantity of MDA was evaluated using a standard curve generated from a solution of 25 µM MDA. The results are expressed as nmoles of MDA per g of dried larvae.

Physico-chemical analysis

The pH was measured on a homogenate (VWR, VDI 25 S41, Radnor, PA, USA) of 1 g of larvae in 10 ml of distilled water using a pH-meter (AB15 pH meter, Accumet BASIC, Thermo Fisher Scientific, Singapore) with an integrated temperature compensation probe (Koniecko, 1984). Also, to evaluate the effectiveness of the drying processes and larval stability in storage, water activity (a_w) was determined on 3 g of ground larvae (Mettler Toledo HR73; AQUA LAB, Guelph, ON, Canada).

The colour of the larvae was measured in triplicate using a chromameter (CR400/410, Konica Minolta Sensing Inc., Osaka, Japan), according to the reflectance coordinates (L*, a*, b*; CIE, 1976) on whole larvae. Colour variation (ΔE^*_{Lab}; Buβler *et al.*, 2015; Joubran *et al.*, 2015; Purschke *et al.*, 2018) was evaluated using Equation 2, for which raw-thawed larvae were used as the reference for pre-treatments, and untreated dry larvae were used as reference for the drying treatments. The colour intensity (chroma, C*) was calculated using Equation 3 and the hue angle (h) was determined by Equation 4 (Hosseinpour *et al.*, 2013):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

$$C^* = \sqrt{\left(a^{*2} + b^{*2}\right)} \quad (3)$$

$$h = \mathrm{Tan}^{-1}\left(b^*/_{a^*}\right) \quad (4)$$

Microbial analysis

Larvae (2 g dried, 25 g raw-thawed) were diluted (1:10) prior to homogenisation in 0.1% (m/v) peptone water (Bacto peptone, Difco Laboratories, Inc., Detroit, MI, USA). To properly disintegrate live larvae, an autoclavable Waring blender (Conair™ Waring™ Laboratory Blenders: Single Speed, Fisher Scientific, Ottawa, ON, Canada; 1 min) with cold peptone water was used. For thawed and dry larvae, homogenisation with a Stomacher (Stomacher® 400C, Seward Laboratory Systems Inc., London, UK) for 1 min at 230 RPM was sufficient. Then, ten-fold dilutions were carried out in 0.1% peptone water for enumeration on the appropriate agar plates (Saucier *et al.*, 2000). Total aerobic mesophilic (TAM) counts (MFHPB-18; Health Canada, 2001a) were performed on plate count agar medium (PCA; Difco Laboratories Inc.; 35 °C for 48 h). Presumptive lactic acid bacteria (LAB) were enumerated on de Man, Rogosa and Sharp petri plates (MRS; Difco Laboratories Inc.; 25 °C for 48 h) in anaerobic conditions (GasPak EZ Anaerobic System, Becton Dickinson [BD], Franklin Lakes, NJ, USA; Saucier *et al.*, 2000). *Enterobacteriaceae* were enumerated and pour plated with violet red bile glucose agar (VRBG, Difco Laboratories Inc; MFLP-43, Health Canada, 1997), and coliforms were enumerated and pour plated with violet red bile agar (VRBA, Difco Laboratories Inc; MFHPB-31, Health Canada, 2001b) after 24 h of incubation at 37 °C. *Escherichia coli* counts were determined on 3M Petrifilm™ plates (3M Food Safety, London, ON, Canada) using method MFHPB-34 (Health Canada, 2013; 37 °C, 18-24 h). Presumptive *Listeria* spp. were determined on PALCAM medium (PALCAM *Listeria* Agars Base; Milipore Sigma, St. Louis, MO, USA) with no supplements, and plates were incubated at 30 °C for 48 h (MFHPB-30, Health Canada, 2011). Yeasts and moulds were enumerated on rose bengal agar (Difco Laboratories Inc.) supplemented with 0.01% chloramphenicol, prepared in 95% ethanol (Xi'an Henrikang Biotech Co., Ltd. GA13183, Shaanxi, China) and incubated at 25 °C for 7 days (MFHPB-22, Health Canada, 2018). Presumptive *Clostridium* spp. were enumerated on reinforced clostridial agar (HiMedia Laboratories, Mumbai, India) and incubated at 36 °C for 48 h under anaerobiosis. *Salmonella* spp. were enumerated on selective CHROMagar™ (CHROMagar, Paris, France) and sent to an external laboratory for detection using Health Canada method MFHPB-20 (2009). Measurements were performed in duplicate. All bacterial counts were transformed to a log value of colony-forming units per g (log cfu/g) prior to statistical analysis conducted according to Gill (2000). The detection limit was used in the statistical analysis when no colonies were detected. This limit was calculated as the value when only one colony was detected on one of the two duplicate plates or Petrifilms.

Storage stability

The larvae that were pretreated in boiling water for 4 min and dried using hot air at 60 °C for 6 h were stored (aliquots of 22 g) in the dark (incubator model 1320, VWR Manufacturing, Inc., Sheldon, OR, USA) in 10-oz sterile bags (WhirlPak, Nasco, Fort Atkinson, WE, USA) at 21.6±0.4 °C for 30 days. On days 0 (n=3), 15 (n=5) and 30 (n=5), microbial analyses were performed as described above. Measurements for pH, a_w, primary and secondary oxidation and colour parameters were also collected.

Statistical analyses

A two-way analysis of variance (ANOVA) was conducted on each physico-chemical and microbial parameter to evaluate the interactions between pre-treatments and drying methods. One-way ANOVAs followed by Tukey HSD tests were subsequently performed to compare the impact of larvae pre-treatments on each drying method (hot-air drying, freeze-drying), as well as to evaluate changes in physico-chemical parameters during storage.

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Although the robustness of the tests could be affected by the small number of samples in this study (Zar, 1999), Shapiro and Bartlett's tests were used to assess normality and homogeneity of the variance, respectively, and should thus be interpreted with caution. When required, log transformations were used for data homogeneity. Experiments and analyses were performed with three or more replicates for each sample and results are expressed as means ± standard deviations. A value of $P<0.05$ indicated a significant difference and $P<0.10$ indicated a tendency. All analyses were performed using the RStudio, version 1.3.959 software package (RStudio, Boston, MA, USA).

3. Results

Physico-chemical and microbial characterisation of BSFL prior to drying

In order to facilitate the drying process and reduce the initial microbial load, larvae were submitted to different pre-treatments, including puncturing, blanching (40 s) and scalding in boiling water for 2, 4, 6 or 8 min. The physico-chemical properties of BSFL were greatly affected by the pre-treatments, drying, and their combined effects, with all interactions being significant ($P<0.05$; Table S1).

Thawed larvae had a near-neutral pH (6.5±0.1) which became alkaline (above 8) after boiling water pre-treatments (Table 1). The largest increases in pH were observed after scalding for 2 and 4 min, reaching a pH of 8.9±0.1 and 8.8±0.2, respectively. Longer periods of pre-treatment in boiling water led to significant reductions in DM, from 22.1±0.1 (thawed larvae) to 18.6±0.7 (8 min). However, ash content was not significantly affected by pre-treatment.

The levels of primary lipid oxidation ranged from 148.6±15.8 to 210.4±39.6 nmol eq. HPC/g, and were not significantly different between larvae pre-treatments. With the exception of the 2-min scalding treatment, levels of secondary lipid oxidation showed significant reductions with longer periods of boiling water pre-treatment ($P=0.001$). Values for TBARS ranged from 58.9±4.1 for raw-thawed larvae to 44.5±2.5 for larvae that were scalded for 8 min.

BSFL became darker ($P<0.001$) after pre-treatments. Levels of lightness (L^*) were least affected by blanching for 40 s and scalding for 6 min. The positive value for a^* indicates that the colour of raw-thawed larvae is in the red spectrum. The intensity of redness was reduced after pre-treatments. Scalding for 2 min resulted in the largest reductions in redness compared to the other pre-treatments ($P<0.001$). The larvae demonstrated the greatest loss in yellowness (b^*) after scalding for 4 min. Overall, colour variations were measured using ΔE, and results indicate that scalding for 2 and 4 min led to the greatest colour difference resulting in brownish-coloured larvae. Blanching for 40 s affected colour variation (ΔE) the least. Colour intensity, C^*, followed a similar pattern to ΔE in being the least affected by blanching for 40 s and most affected by scalding for 2 and 4 min ($P<0.001$). Compared to the control and the other treatments, scalding for 2 min resulted in the highest hue angle (h^*; $P<0.001$), leading to colours that fall within the yellow-green spectrum.

Larvae pre-treatments significantly affected their microbial load ($P<0.001$; Table S2). Larvae were punctured to improve drying efficiency. Puncturing increased the microbial load for all the microbial groups that were tested, with the exception of TAM (Table 2). However, all the increases were less than one log unit and are therefore of little practical value. Microbial loads were greatly reduced with longer periods of pre-treatment in boiling water ($P<0.05$) for all tested microbial groups. All the scalding treatments yielded microbial load log reductions greater than 1 for all the tested microbial groups, whereas TAM was the only microbial group to achieve a 1-log reduction after 40 s of blanching (Table 2).

Drying kinetics

Drying kinetics curves (i.e. X/Xo vs time) are presented in Figure 1 and 2 for hot-air drying and freeze-drying, respectively. These curves show a characteristic decrease in water content over the drying period, i.e. a negative exponential tendency with a marked decrease in water content in the beginning of the process, followed by a decline in the dehydration rate when bound water becomes harder to remove. Towards the end of the drying period, a plateau forms, which indicates an equilibrium in mass transfer between the product and the drying environment.

As expected, Figure 2 shows that freeze-drying kinetics were slow (14-72 h to complete drying depending on the larval pre-treatment) compared to hot-air drying (4-12 h, Figure 1). Fourteen hours of freeze-drying were required to reach a water content of 0.1 g water/g DM when larvae were first scalded for 8 min, while more than 72 h were required for the control samples (no pre-treatment).

Optimal drying times for achieving similar relative water contents were estimated from the drying kinetics curves in Figure 1 and 2. Using these curves, reductions in drying time as a result of pre-treatments were calculated (Table 3). Pre-treatments showed a marked impact on the time required to dry the larvae. For instance, larvae that were scalded for 8 min required just 4 hours to decrease their initial moisture content to 0.1 g water/g dry mass by hot-air drying, a 66.7% reduction compared to the time needed for those that did not undergo any pre-treatments (Table 3). For freeze-drying, an 80.6% drying time reduction was achieved after an 8-min scalding pre-treatment.

Table 2. Microbial counts (log cfu/g) from black soldier fly larvae after pre-treatment in boiling water.[1]

Parameters[2]	Raw thawed	Raw thawed	Punctured	Blanching	Scalding			
				40 s	2 min	4 min	6 min	8 min
	WB[3]	DB[3]	DB	DB	DB	DB	DB	DB
TAM	8.28±0.01	8.96±0.01[a]	8.38±0.00[b] (0.58)	7.90±0.02[c] (1.06)	7.78±0.01[d] (1.18)	7.27±0.02[e] (1.39)	6.92±0.01[f] (2.04)	6.72±0.05[g] (2.24)
LAB	7.71±0.15	8.23±0.51[b]	8.47±0.02[a] (-0.24)	7.56±0.02[c] (0.67)	7.07±0.06[d] (1.16)	6.76±0.06[e] (1.47)	5.85±0.05[f] (2.38)	5.54±0.06[g] (2.69)
Enterobacteriacea	6.60±0.23	6.92±0.03[a]	7.07±0.02[a] (-0.15)	6.47±0.01[b] (0.45)	5.85±0.02[c] (1.07)	5.71±0.03[c] (1.21)	5.40±0.09[d] (1.52)	5.30±0.13[d] (1.62)
Coliforms	6.59±0.26	6.92±0.04[b]	7.09±0.02[a] (-0.17)	6.45±0.02[c] (0.47)	5.89±0.06[d] (1.03)	5.73±0.04[e] (1.19)	5.53±0.04[f] (1.39)	5.03±0.04[g] (1.89)
Listeria spp.	6.49±0.07	7.47±0.03[b]	7.87±0.04[a] (-0.40)	6.85±0.10[c] (0.62)	6.24±0.07[d] (1.23)	5.85±0.05[e] (1.62)	5.54±0.12[e] (1.93)	5.67±0.05[e] (1.80)

[1] Data represent means ± standard deviations (n=9). Data presented in parentheses represent the logarithmic reduction between the raw-thawed larvae and the pre-treated larvae on a dry basis. Values with different letters in the same row are significantly different ($P<0.05$).

[2] LAB = lactic acid bacteria; TAM = total aerobic mesophilic counts.

[3] DB = dry basis; WB = wet basis. Results are expressed on a dry basis in order to take into account the concentration effect of drying. Multiple comparisons were therefore performed with values expressed as a percentage (%) of the dry matter (DB).

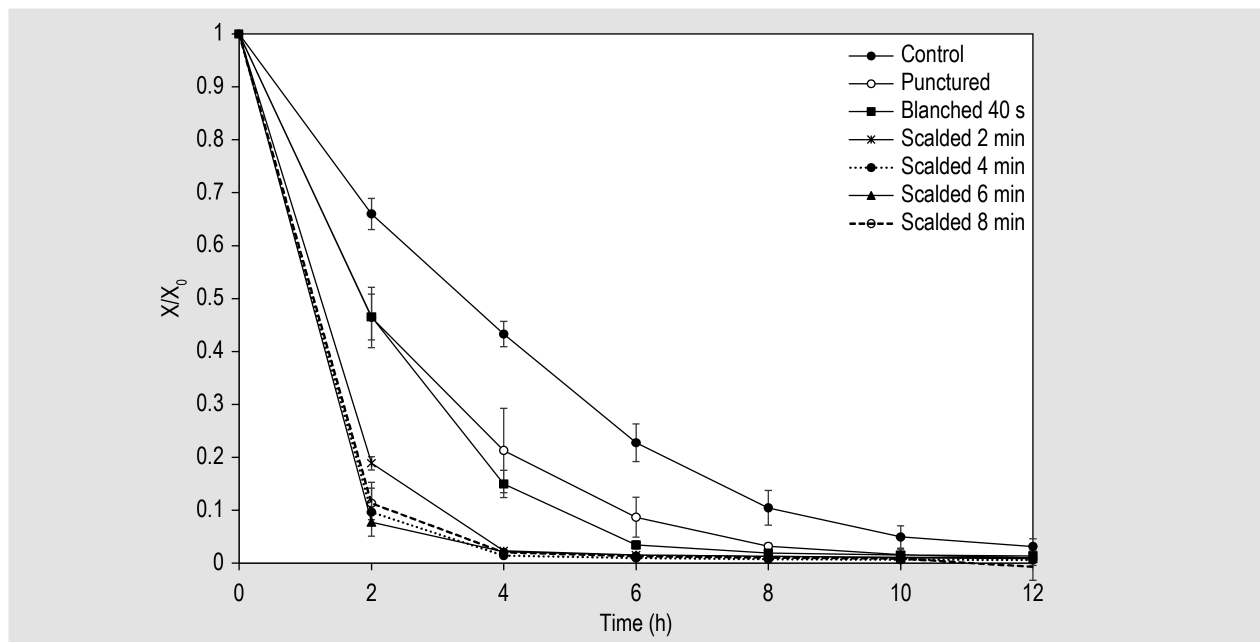

Figure 1. Kinetics curves for hot-air drying (60 °C) black soldier fly larvae after different pre-treatments. Drying experiments were done in triplicate (n=3).

Overall, puncturing the surface of larvae or treating the samples in boiling water accelerated the drying process. With hot-air drying, a 16 to 66% drying reduction time was observed. A 66 to 80% reduction was observed from 8 min of scalding prior to hot-air drying and freeze-drying, respectively (Table 3). These results indicate that the larvae pre-treatments facilitate water loss during BSFL drying.

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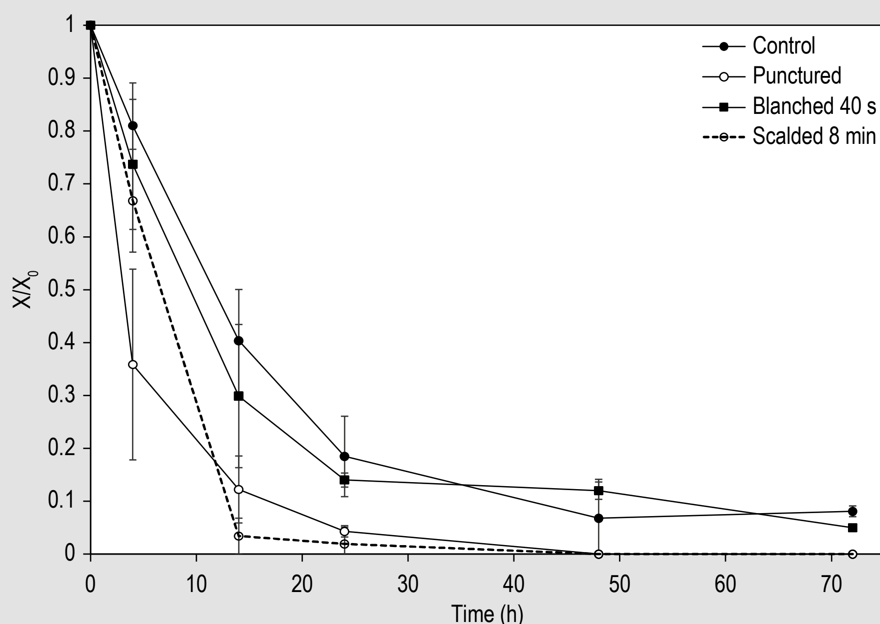

Figure 2. Kinetics curves for freeze-drying (40 °C heating plate) black soldier fly larvae after different pre-treatments. Drying experiments were done in triplicate (n=3).

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Table 3. Optimal drying times, drying time reductions and final water content.

Treatments		Drying times (h)	Drying time reductions (%)	Relative water content, X/Xo
Hot-air drying				
Control[1]		12	---	0.03
Punctured		10	16.7	0.02
Blanched	40 s	8	33.3	0.02
Scalded	2 min	6	50.0	0.02
	4 min	6	50.0	0.02
	6 min	6	50.0	0.02
	8 min	4	66.7	0.01
Freeze-drying				
Control[1]		72	---	0.05
Punctured		24	66.7	0.03
Blanched	40 s	48	33.3	0.03
Scalded	8 min	14	80.6	0.03

[1] Control = no pre-treatment. Drying curves were done in triplicate (n=3).

Physico-chemical and microbial characterisation of dried BSFL

Physico-chemical properties of hot-air dried larvae are presented in Table 4 and those for freeze-dried larvae are presented in Table 5. Water activity varied from 0.24 to 0.37 and 0.26 to 0.37 for hot-air and freeze-dried larvae, respectively, suggesting that water removal was efficient for all cases. The DM content was above 90% for all larvae that were pre-treated with boiling water and subsequently dried using either drying method. The DM content was less than or close to 90% for the raw-thawed or punctured and dried larvae. Based on the DM content, puncturing improves drying performance when using freeze-drying, but not when using hot air.

When larvae were dried with hot air, the ash content decreased significantly when scalding pre-treatments were ≥4 min. However, when larvae were freeze-dried, the ash content was the highest for blanched dried larvae and the lowest for the raw-thawed dried larvae. Hot-air drying resulted in the lowest protein content for larvae that underwent 4 min of scalding, which however, was not significantly different from those that were blanched for 40 s ($P<0.05$). Overall, the protein content variation was ≤4.6% for larvae dried with hot air. The protein content for raw-thawed freeze-dried larvae and those scalded for 8 min were similar, but both groups of larvae had higher protein content compared to those that were punctured and those that were blanched for 40 s prior to freeze-drying ($P<0.05$). However, protein content variation was no more than 1.5% among freeze-dried larvae. The lipid content for hot-air dried larvae was the highest among those that were scalded for 4 min (29.1±0.4%) and the lowest for punctured larvae (18.7±2.2%). Among freeze-dried larvae, those that were punctured also had the lowest lipid content (18.7±0.5%), and the lipid content never exceeded 23.3±0.1% for any of the other freeze-dried larvae.

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Table 4. Physico-chemical properties of black soldier fly larvae after hot-air drying (60 °C; X/X0<0.1).[1]

Parameters[2]	Hot-air drying						
	Raw thawed dried[3]	Punctured	Blanching	Scalding			
			40 s	2 min	4 min	6 min	8 min
a_w	0.35±0.01[a]	0.34±0. 01[ab]	0.24±0.01[c]	0.37±0.05[a]	0.31±0.01[ab]	0.29±0.01[b]	0.29±0.01[b]
DM (%)	88.1±1.1[e]	82.0±3.3[f]	94.6±0.2[ab]	91.7±0.7[cd]	95.1±0.2[a]	93.3±0.2[abc]	90.8±1.5[cd]
Ash (%)	10.4±0.1[a]	10.1±0.4[a]	9.2±0.1[ab]	9.1±0.1[ab]	8.2±0.1[b]	8.7±0.1[b]	9.0±0.4[b]
Proteins (%)	40.0±1.1[a]	38.7±1.0[ab]	36.5±0.3[cd]	37.7±0.6[abc]	35.4±0.1[d]	38.0±0.3[abc]	37.5±1.2[bc]
Lipids (%)	24.3±0.4[b]	18.7±2.2[c]	25.0±0.3[ab]	23.8±1.0[b]	29.1±0.4[a]	24.4±0.5[b]	25.9±3.5[a]
XO (nmol eq. HPC/g)	1,427.7±48.1[b]	1,412.7±103.3[b]	488.9±14.4[cd]	473.1±40.1[d]	368.5±30.4[d]	603.9±41.7[c]	1,934.9±178.7[a]
TBARS (nmol mda/g)	142.2±3.8[a]	132.5±7.8[a]	54.3±2.1[cd]	61.4±7.5[c]	43.0±1.1[d]	66.2±5.2[c]	91.3±2.8[b]
L*	25.3±4.9[bc]	22.9±1.7[c]	40.8±1.1[a]	28.4±4.8[bc]	32.4±2.9[ab]	35.2±3.3[ab]	34.1±2.3[ab]
a*	3.6±0.2[a]	2.0±0.1[b]	4.0±0.6[a]	3.7±0.6[a]	3.8±0.3[a]	3.1±0.3[ab]	2.4±0.3[b]
b*	8.3±0.3[d]	5.2±0.2[e]	19.0±0.9[a]	14.0±0.5[b]	13.4±0.2[b]	13.9±1.2[c]	10.8±1.3[d]
ΔE[4]	Reference	4.4±1.2[c]	18.9±1.3[a]	7.2±3.0[b]	8.9±2.4[b]	11.5±3.1[b]	9.4±1.8[b]
C*	9.1±0.2[c]	5.6±0.3[cd]	19.5±1.0[a]	14.5±0.7[a]	13.9±0.3[ab]	14.2±1.2[a]	11.1±1.3[ab]
h*	66.4±1.5[b]	68.8±0.6[b]	78.2±1.2[a]	75.3±1.9[a]	74.2±1.0[a]	77.5±0.6[a]	77.3±3.2[a]

[1] Data represent means ± standard deviations (n=3). Values with different letters in the same row are significantly different (*P*<0.05).

[2] a_w = water activity, CHP = cumene hydroperoxide; DM = dry matter, eq. = equivalent; MDA = malonaldehyde; results TBARS = thiobarbituric acid reactive substances; XO = xylenol orange; are expressed as a percentage (%) of the dry matter.

[3] Live larvae euthanised by freezing at -40 °C and then thawed prior to drying.

[4] $\Delta E = [(L^*_{ref.}-L^*_{treated})^2 + (a^*_{ref}-a^*_{treated})^2 + (b^*_{ref}-b^*_{treated})^2]^{1/2}$ indicates the colour difference using raw-thawed larvae as the reference. Reference larvae were assigned a value of zero. Chroma value $C^* = (a^{*2}+b^{*2})^{1/2}$ measures colour intensity. Hue angle $h = Tan^{-1}(b^*/a^*)$ measures colour appearance.

Table 5. Physico-chemical properties of black soldier fly larvae after freeze-drying (40 °C; X/X0<0.1).[1]

Parameters[2]	Freeze-dried			
	Raw-thawed dried[3]	Punctured	Blanching	Scalding
			40 s	8 min
a_w	0.37±0.02[a]	0.28±0.01[c]	0.26±0.01[c]	0.33±0.01[b]
DM (%)	85.8±1.5[c]	89.7±0.9[b]	92.6±0.8[a]	91.3±0.5[a]
Ash (%)	8.8±0.1[d]	9.4±0.1[b]	10.0±0.1[a]	9.1±0.1[c]
Proteins (%)	38.5±0.5[a]	37.1±0.3[b]	37.3±0.2[b]	38.6±0.5[a]
Lipids (%)	23.3±0.5[a]	18.7±0.5[b]	20.7±1.9[ab]	21.7±0.8[a]
XO (nmol eq. HPC/g)	1,937.7±132.1[a]	1,213.8±36.3[b]	1,162.7±89.5[b]	596.9±56.9[c]
TBARS (nmol MDA/g)	62.2±4.8	80.0±4.4	65.8±13.9	58.8±11.0
L*	43.4±1.9[b]	56.1±0.6[a]	47.0±2.7[b]	59.8±5.3[a]
a*	3.9±0.5[a]	1.8±0.1[c]	3.0±0.2[b]	3.9±0.1[a]
b*	17.7±0.2[a]	16.7±0.1[ab]	14.9±1.4[bc]	13.5±0.1[c]
ΔE[4]	Reference	12.9±0.6[a]	4.8±2.8[b]	17.0±5.2[a]
C*	18.1±0.3[a]	16.8±0.1[a]	15.2±1.4[b]	14.0±0.1[c]
h*	77.6±1.5[b]	83.8±0.1[a]	78.7±0.5[b]	74.0±0.2[c]

[1] Data represent means ± standard deviations (n=3). NS = not significant. Values with different letters in the same row are significantly different (*P*<0.05).

[2] a_w = water activity; CHP = cumene hydroperoxide; DM = dry matter; eq. = equivalent; MDA = malonaldehyde; TBARS = thiobarbituric acid reactive substances; XO = xylenol orange; results are expressed as a percentage (%) of the dry matter.

[3] Live larvae euthansed by freezing at -40 °C and then thawed prior to drying.

[4] $\Delta E = [(L^*_{ref.}-L^*_{treated})^2 + (a^*_{ref}-a^*_{treated})^2 + (b^*_{ref}-b^*_{treated})^2]^{1/2}$ indicates the colour difference using raw-thawed larvae as the reference. Reference larvae were assigned a value of a zero. Chroma value $C^* = (a^{*2}+b^{*2})^{1/2}$ measures colour intensity. Hue angle $h = Tan^{-1}(b^*/a^*)$ measures colour appearance.

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Among larvae that were dried with hot air, the highest levels of primary oxidation were observed for larvae scalded for 8 min (1,934.9±178.7 nmol eq. CHP/g), followed by raw-thawed and punctured larvae (1,427.7±48.1 and 1,412.7±103.3 nmol eq. CHP/g, respectively) and then larvae scalded for 6 min (603.9±41.7 nmol eq. CHP/g). The lowest primary oxidation levels observed were ≤488.9±14.4 nmol eq. CHP/g for larvae that were blanched for 40 s and scalded for 2 and 4 min. These low values are nearly four times lower than the highest level obtained using this drying method (Table 4). Primary oxidation was markedly lower in freeze-dried larvae that were scalded for 8 min. Indeed, primary oxidation was three times lower compared to raw-thawed freeze-dried larvae and two times lower compared to larvae subjected to the two other pre-treatments (Table 4).

Scalding treatments were beneficial for reducing secondary oxidation levels among larvae dried by hot air, compared to the control (Table 4). TBARS values were the lowest among larvae scalded for 4 min followed by those blanched for 40 s prior to hot-air drying ($P<0.05$). With freeze-drying, secondary oxidation levels were not significantly different among treatments ($P>0.05$; Table 5).

Figure 3A presents the lipid oxidation results (TBARS) for some larvae pre-treatments and drying methods. As shown in this figure, 8 min of scalding caused a slight decrease in the oxidation index compared to raw-thawed larvae (44.5±2.5 compared to 58.9±4.1 nmol MDA/g, respectively). However, TBARS levels for the control larvae after hot-air drying increased substantially to 142.2±3.8 nmol MDA/g, due to the extended drying time required for non pre-treated larvae to reach dryness (12 hours, Table 3). This extended drying time allows prolonged contact between the samples and oxygen at 60 °C. Scalding pre-treatment prior to air-drying led to reductions in secondary oxidation in dried larvae, compared to the control (Figure 3A). However, TBARS levels for hot-air dried larvae increased significantly with increasing scalding times during pre-treatment. Thus, longer scalding times may be beneficial for reducing the drying time that is required when using hot air (Table 3). However, depending on the scalding time, pre-treatment in boiling water may also decrease the lipid quality in the dried larvae through oxidation (Figure 3A).

Freeze-dried larvae (control) presents oxidation indexes similar to raw-thawed larvae (Figure 3A) due to the absence of oxygen during freeze-drying under vacuum. The 8-min scalding pre-treatment reduced freeze-drying time by 80.6% (Table 3) without compromising the lipid quality of the product (Figure 3A).

Larvae pre-treatments in boiling water significantly reduced the darkening effect of hot-air drying (Figure 4). The L* values for these pre-treated larvae were higher than those for raw-thawed (control) and punctured larvae ($P<0.05$). Blanching for 40 s presented the lightest-coloured larvae (Table 4). Among larvae that were freeze-dried, the darkest colours were observed among raw-thawed (control) and 40-s blanched larvae ($P<0.05$; Table 5). As expected, freeze-drying had a weaker darkening effect than hot-air drying, as all L* values were higher with freeze-drying than hot-air drying for the same pre-treatments. Punctured dried larvae yielded L* values that were two times greater for freeze-drying compared to hot-air drying.

Redness (a*) for hot-air dried larvae was the highest among the raw-thawed controls as well as those that were blanched for 40 s and scalded for 2, 4 and 6 min. The lowest levels of redness were observed in the punctured and 8-min scalded larvae ($P<0.05$; Table 4). When punctured larvae were freeze-dried, redness was at its lowest, at 1.8±0.1. Redness levels were at their highest among raw-thawed larvae and 8-min scalded larvae (Table 5). Levels of yellowness (b*) were highest among hot-air dried larvae that were blanched for 40 s, at 19.1±0.9. Yellowness was the lowest among punctured larvae, at 5.2±0.2. Scalded larvae exhibited intermediate yellowness, with a greater loss of yellowness as the pre-treatment time in boiling water increased (Table 4). The decrease in yellowness was less marked with freeze-dried larvae, and scalding for 8 min led to the largest reductions in yellowness (Table 5).

Among the larvae pre-treatments that were tested, puncturing resulted in the least amount of colour change (ΔE) compared to raw-thawed larvae when subjected to hot-air drying. Among those treated in boiling water, blanching for 40 s showed the greatest colour change (Table 4). Freeze-dried samples that were blanched for 40 s showed the least colour change compared to the raw-thawed larvae. The greatest colour change was observed in those that were scalded for 8 min and those that were punctured (Table 5). Larvae that were freeze-dried showed significant reductions in colour intensity (C*) with increasing pre-treatment severity compared to those that were raw-thawed (control; $P<0.001$). Therefore, colour intensity among freeze-dried larvae was at its lowest after scalding for 8 min. Hue angles were similar for all larvae pre-treated in boiling water, and were higher than the raw-thawed and punctured hot-air dried larvae (Table 4). For the freeze-dried larvae, hue angles were highest among punctured larvae and the lowest among those that were scalded for 8 min (Table 5).

Figure 3B shows larval colour changes using the initial colour of raw-thawed untreated larvae as the reference. Colour differences can be classified as a function of ΔE values. If $\Delta E>3$, the colour difference is very visible, $1.5<\Delta E<3$ means that there is some difference, and $\Delta E<1.5$ indicates a negligible difference (Adekunte *et al.*, 2010; Purschke *et al.*, 2018). Therefore, as shown in Figure 3B, all

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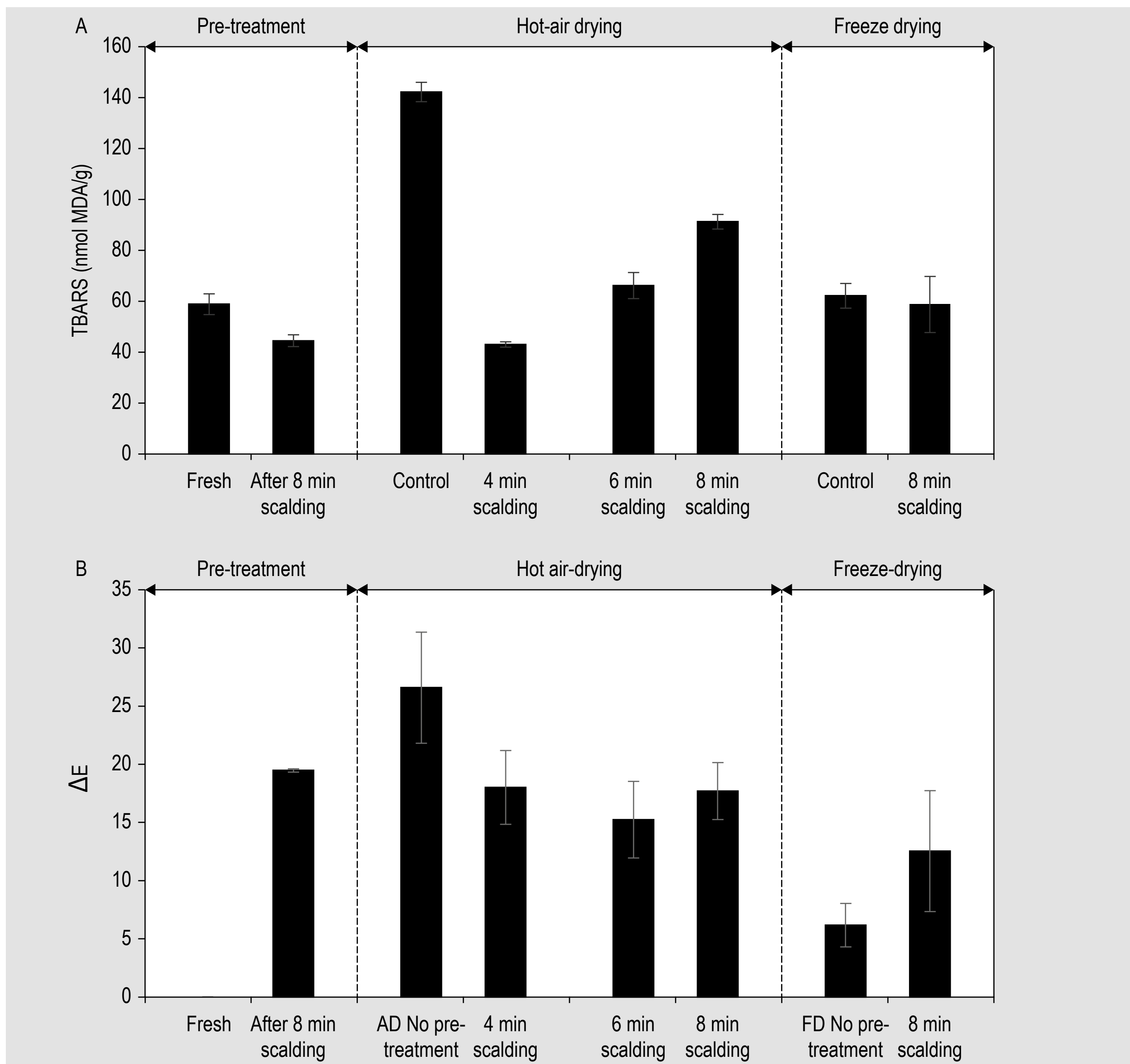

Figure 3. Impact of selected larvae pre-treatments and drying methods on (A) secondary lipid oxidation and (B) colour. The raw-thawed larvae were used as the reference for ΔE calculations. Drying experiments were done in triplicate (n=3).

applied treatments (pre-treatments and drying methods) caused major colour changes in the larvae.

Hot-air drying alone reduced microbial counts by 3.56 to 4.35 log compared with raw-thawed larvae, as presented in Table 6. As expected, microbial counts significantly decreased with increasing scalding times. Log reductions >4 were achieved for all cell counts when larvae were pre-treated with 6 and 8 min of scalding, compared to those that were raw-thawed. However, larvae pre-treatments appear to have limited beneficial effects on cell counts. Microbial counts that were significantly reduced compared to raw-thawed dried larvae required pre-treatment scalding times that were between 4 and 8 min, depending on the microbial type. This was true with the exception of *Clostridium* spp., yeasts and moulds, for which all pre-treatments were effective in reducing microbial load ($P<0.05$). A log reduction greater than one, compared to the raw-thawed dried larvae, was only obtained for TAM, LAB and yeasts and moulds.

Freeze-drying also reduced the various microbial counts compared to raw-thawed larvae, but not to the same extent as hot-air drying. Log reductions for the various microbial counts after freeze-drying ranged from 1.29 to 2.69 (Table 7). Larvae pre-treatment in boiling water significantly reduced the microbial counts, while puncturing had little effect compared to raw-thawed freeze-dried larvae. This was the case for all the microbial group tested with the exception of LAB, *Listeria* spp., *E. coli*, presumptive *Clostridium*

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Figure 4. Visual aspect of black soldier fly larvae after pre-treatment followed by hot-air drying (A = raw-thawed dried; B = punctured; C = blanching 40 s; D, E, F and G = scalding for 2, 4, 6 and 8 min, respectively) or freeze-drying (H = raw-thawed dried; I = punctured; J = blanching 40 s; K = scalding for 8 min).

Table 6. Microbial counts (log cfu/g) from black soldier fly larvae after hot-air drying (60 °C; X/X0 <0.1).[1]

	After hot-air drying							
	Raw thawed, DB[3]	Raw thawed dried, DB[3]	Punctured DB	Blanching	Scalding			
Parameters[2]				40 s	2 min	4 min	6 min	8 min
TAM	8.95±0.01	5.39±0.0[ab] (3.56)	5.47±0.00[a] (3.48)	5.29±0.01[ab] (3.66)	5.18±0.01[b] (3.77)	4.92±0.01[c] (4.00)	4.52±0.02[d] (4.43)	4.21±0.08[e] (4.74)
LAB	8.25±0.03	4.50±0.12[ab] (3.75)	4.74±0.04[a] (3.51)	4.64±0.21[a] (3.61)	4.58±0.02[ab] (3.67)	4.35±0.05[bc] (3.90)	4.12±0.06[c] (4.13)	3.46±0.05[d] (4.79)
Enterobacteriacea	6.94±0.04	3.31±0.02[a] (3.63)	3.69±0.05[a] (3.25)	3.78±0.01[a] (3.16)	3.37±0.01[ab] (3.57)	3.24±0.02[ab] (3.69)	2.83±0.03[b] (4.11)	2.71±0.05[b] (4.23)
Coliforms	6.92±0.04	3.25±0.02[c] (3.67)	3.52±0.07[b] (3.40)	3.71±0.03[a] (3.21)	3.20±0.00[c] (3.72)	3.17±0.01[c] (3.75)	2.84±0.05[d] (4.08)	2.56±0.08[e] (4.36)
Listeria spp.	7.48±0.03	3.55±0.03[bc] (3.93)	4.12±0.01[a] (3.36)	4.10±0.01[a] (3.38)	3.55±0.07[bc] (3.93)	3.64±0.11[b] (3.84)	3.40±0.07[c] (4.08)	2.92±0.03[d] (4.56)
Escherichia coli	7.19±0.50	2.84±0.04[cd] (4.35)	3.49±0.02[b] (3.70)	3.65±0.02[a] (3.54)	3.27±0.02[c] (3.92)	2.79±0.08[d] (4.40)	2.61±0.02[e] (4.58)	2.36±0.10[f] (4.83)
Clostridium spp.[4]	ND	5.19±0.01[a]	5.04±0.00[b]	4.88±0.00[c]	4.69±0.01[d]	4.50±0.01[e]	4.30±0.02[f]	4.11±0.02[g]
Yeasts and molds[5]	ND	4.83±0.00[a]	4.67±0.04[b]	4.46±0.03[c]	4.36±0.03[c]	4.09±0.03[d]	3.41±0.07[e]	2.92±0.07[f]

[1] Data represent means ± standard deviations (n=9) for raw-thawed larvae and n=3 for dried larvae, ND = not determined. Data presented in parentheses are the logarithmic reductions between the raw-thawed larvae and pre-treated larvae on a dry basis. Values with different letters in the same row are significantly different ($P<0.05$).

[2] LAB = lactic acid bacteria; TAM = total aerobic mesophilic counts.

[3] DB = dry basis.

[4] Analyses were performed only on dry larvae. *Clostridium* spp. are presumptive data as no confirmation tests were conducted.

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as well as yeasts and moulds, though log variations were all less than 1. Scalding for 8 min was the most effective pre-treatment for reducing microbial counts compared to raw-thawed larvae, yielding a reduction of 3.71 to 4.95 log. Among the freeze-dried larvae, pre-treatments in boiling water were most efficient for reducing microbial load, with the drying process having only a minor effect.

Storage stability

The powder obtained from ground BSFL after thawing, scalding for 4 min and hot-air drying (60 °C) was stored for 30 d at room temperature to assess it stability. Water activity remained stable over the storage period, but the pH became slightly more acidic by day 30 ($P<0.05$; Table 8). Primary and secondary oxidation increased significantly over time ($P<0.05$), while none of the colour parameters changed significantly over time. Nonetheless, ΔE values fell between 1.5 and 3, so colour variations were therefore deemed perceptible. However, the colour variation in samples collected at specific times were similar to those observed between times (data not shown), suggesting a certain level of colour heterogeneity within each sampling time.

Overall, microbial counts decreased significantly ($P<0.05$) over the storage period except for yeasts and mould. Counts for this group remained at the same levels (Table 9). However, all variations were less than one log unit. *Salmonella* counts were below the detection level (1.70 cfu/g) using the selective CHROMagar. The absence of *Salmonella* was confirmed by an external laboratory using enrichment procedures (Health Canada, 2009).

4. Discussion

BSFL are good candidates for upcycling wet residual organic matter and can lead to the reintroduction of valued nutrients into farming systems to raise livestock in a more sustainable manner (Gold *et al.*, 2018; Lalander *et al.*, 2019). The bioconversion of residual organic matter using insects has real potential for future approaches in organic waste management. If properly processed, these insects can be used as a stable feed ingredient.

Drying process

As expected, our results indicate that whole dry larvae can be obtained faster when hot-air drying is used, compared to freeze-drying (Table 3). The drying process can be 2.5 to 6 times faster, depending on the larvae pre-treatment that is applied. Purschke *et al.* (2018) studied the dry fractionation of blanched *Tenebrio molitor* L. larvae. In their study, samples were dehydrated for 24 hours in a convection oven at 60 °C and for 48 hours, or twice as long, in a freeze-dryer. Although Purschke *et al.* (2018) did not investigate drying kinetic optimisation, as in this study, their results suggest that freeze-drying times are often longer than air-drying times. Freeze-drying mainly uses the sublimation phenomenon to eliminate most of the water in a product (primary drying). After most of the ice has been sublimated, desorption of non-freezable water occurs (secondary drying). Thus, water loss is first controlled by the progression of a receding sublimation front, leaving a porous dry layer that increases as drying proceeds (Ratti, 2010). A major portion of the bound water is in unfrozen

Table 7. Microbial counts (log cfu/g) from black soldier fly larvae after freeze-drying.[1]

Parameters[2]	After freeze-drying				
	Raw thawed, DB[3]	Raw thawed dried, DB[3]	Punctured, DB	Blanching 40 s DB	Scalding 8 min DB
TAM	8.97±0.01	7.46±0.00^a (1.51)[4]	7.47±0.01^a (1.50)	5.29±0.01^b (3.68)	4.96±0.01^c (4.01)
LAB	8.26±0.00	6.97±0.01^a (1.29)	6.72±0.03^b (1.54)	4.58±0.04^c (3.68)	4.55±0.02^c (3.71)
Enterobacteriacea	6.90±0.01	5.35±0.05^a (1.55)	5.44±0.07^a (1.46)	3.21±0.05^b (3.69)	3.03±0.02^c (3.87)
Coliforms	6.92±0.02	5.24±0.05^a (1.68)	5.33±0.07^a (1.59)	3.11±0.04^b (3.81)	3.03±0.02^b (3.89)
Listeria spp.	7.49±0.04	6.34±0.01^a (1.15)	6.16±0.03^b (1.33)	4.06±0.06^c (3.43)	3.41±0.06^d (4.08)
Escherichia coli	7.86±0.02	5.17±0.04^a (2.69)	5.36±0.02^b (2.50)	3.13±0.02^c (4.73)	2.91±0.03^d (4.95)
Clostridium spp.[4]	ND	5.02±0.01^a	4.95±0.01^b	4.90±0.01^c	4.20±0.03^d
Yeasts and moulds	ND	4.90±0.01^a	4.71±0.03^b	4.49±0.01^c	3.03±0.05^d

[1] Data represent means ± standard deviations (n=3), ND = not determined. Data presented in parentheses are the logarithmic reductions between the raw-thawed and pre-treated larvae based on a dry basis. Values with different letters in the same row are significantly different ($P<0.05$).

[2] LAB = lactic acid bacteria; TAM = total aerobic mesophilic counts.

[3] DB = dry basis.

[4] Analyses were performed only on dry larvae. *Clostridium* spp. are presumptive data as no confirmation tests were conducted.

Table 8. Physico-chemical properties of black soldier fly larvae after thawing, scalding for 4 min and hot-air drying (6 h at 60 °C) during storage (30 d) at room temperature (21 °C).[1]

Parameters[2]	Storage time		
	Day 0	Day 15	Day 30
pH	7.7±0.1[a]	7.6±0.1[a]	7.4±0.1[b]
a_w	0.33±0.00	0.32±0.00	0.34±0.01
XO (nmol eq. CHP/g)	554.8±28.2[a]	768.4±45.8[b]	861.2±61.1[c]
TBARS (nmol MDA/g)	59.5±4.3[a]	66.4±4.1[b]	72.7±1.3[c]
L*	32.1±1.3	31.1±2.7	31.7±2.1
a*	3.5±0.2	3.6±0.1	3.3±0.2
b*	13.4±0.7	13.5±1.0	12.2±1.0
ΔE[3]	Reference	2.5±1.7	2.2±1.3
C*	13.9±0.8	14.1±1.4	12.7±1.0
h*	75.0±0.5	75.0±1.7	74.7±1.8

[1] Data represent means ± standard deviations; n=3 on day 0 and n=5 on days 15 and 30. NS = not significant. Values with different letters in the same row are significantly different (P<0.05).

[2] a_w = water activity, CHP = cumene hydroperoxide; eq. = equivalent; MDA = malonaldehyde; TBARS = thiobarbituric acid reactive substances; XO = xylenol orange; results are expressed as a percentage (%) of the dry matter.

[3] $\Delta E = [(L^*_{ref.}-L^*_{treated})^2 + (a^*_{ref}-a^*_{treated})^2 + (b^*_{ref}-b^*_{treated})^2]^{1/2}$ indicates the colour difference using Day 0 as the reference. The reference is assigned a value of zero. Chroma value $C^* = (a^{*2}+b^{*2})^{1/2}$ measures colour intensity. Hue angle $h = Tan^{-1}(b^*/a^*)$ measures colour appearance.

Table 9. Microbial counts (log cfu/g) from black soldier fly larvae during storage (30 d) at room temperature (21 °C) after thawing, scalding for 4 min and hot-air drying (6 h at 60 °C).[1]

Parameters[2]	Storage time		
	Day 0	Day 15	Day 30
TAM	4.94±0.01[a]	4.83±0.02[b]	4.80±0.01[b]
LAB	4.33±0.04[a]	4.16±0.08[b]	4.06±0.04[c]
Enterobacteriacea	3.24±0.04[a]	3.03±0.03[b]	3.05±0.06[b]
Coliforms	3.14±0.02[a]	3.08±0.04[b]	3.02±0.02[c]
Listeria spp.	3.53±0.12[a]	3.13±0.13[b]	3.09±0.09[b]
Escherichia coli	2.83±0.13[a]	2.51±0.15[b]	2.43±0.13[b]
Clostridium spp.	4.55±0.03[a]	4.45±0.07[ab]	4.40±0.04[b]
Yeasts and moulds	4.03±0.02	4.04±0.01	4.05±0.01
Salmonella	BDL[3]	BDL	BDL

[1] Data represent means ± standard deviations; n=3 on day 0 and n=5 on days 15 and 30. Values with different letters in the same row are significantly different (P<0.05).

[2] LAB = lactic acid bacteria; TAM = total aerobic mesophilic counts.

[3] BDL = below detection level (1.70 log cfu/g). Absence of *Salmonella* was confirmed by an external laboratory using method MFHPB-20 from Health Canada (2009).

state in this dry layer and the freeze-drying rate becomes very slow during secondary drying (Vega-Mercado *et al.*, 2001). Both primary and secondary drying stages during freeze-drying lead to slower drying kinetics than convective drying, even at a 40 °C shelf plate.

For both drying methods, larvae pre-treatments were effective in reducing drying times. Drying times were reduced by 16 to 66% and 66 to 80% for hot-air and freeze-drying, respectively (Table 3). Puncturing holes in the larval cuticle alone provided 16.7 and 66.7% reductions in drying time for hot-air and freeze-drying, respectively (Table 3). Treating larvae with boiling water for various lengths of time is also likely to damage the cuticle. The cuticle is composed of a thin, outer protein layer called the epicuticle, and a thick, inner, chitin-protein layer called the procuticle (Barnes, 2020). In insects, the external epicuticle structure contains wax (Boevé *et al.*, 2004), which serves as a water-impermeable barrier, protecting larvae against desiccation (Andersen, 2009). Thus, pre-treatments such as puncturing or melting the wax by scalding or directly steaming may facilitate insect larvae drying. Similar observations have been made in berry dehydration, where the waxy cuticle slowed down water loss during drying due to its hydrophobicity (Araya-Farias *et al.*, 2011; Ketata *et al.*, 2013). Scanning electron microscopy and optical microscopy of berry skins have demonstrated that removing the waxy cuticle layer after pre-treatment facilitated water loss during drying (Ketata *et al.*, 2013).

Microbial load reduction

Initial microbial load levels for TAM and *Enterobacteriacea* in the raw-thawed larvae in this study were above the levels reported by Kashiri *et al.* (2018) and Cappellozza *et al.* (2019), but below those reported by Larouche *et al.* (2019) and Wynants *et al.* (2019) for BSFL raised with different substrates. The larvae in our experiment were punctured as a way to ease water removal during larvae drying. Although this was effective in reducing the drying time, puncturing larvae prior to drying increased the microbial load. All microbial counts were significantly higher for punctured larvae compared to raw-thawed larvae, with the exception of TAM. However, all the log variations were below one and could simply be due to the result of an improved extraction by the puncture of the larvae structure (Table 2). Similarly, and independent of the drying method used, when comparing punctured dried larvae to raw-thawed dried larvae with no pre-treatment, significant differences can be observed among the various microbial groups tested. Notably, *E. coli* counts increased significantly whereas *Clostridium* spp. and yeast and moulds decreased significantly. All the significant variations observed, however, were less than 1 log unit (Tables 6 and 7). Any additional

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processing step that does not have an inhibitory effect on the microbiome could potentially introduce contamination. However, a log variation equal to or greater than one is the threshold practical value for the assessment of processing microbial reduction efficacy (Gill, 2000).

The significant interactions observed between larvae pre-treatments and drying methods for all the tested microbial groups, except for yeasts and moulds (Table 3), suggest that microbial reduction varies depending on the drying method used, when the same pre-treatments are applied. Heat treatments are commonly used to reduce microbial populations in food and feed. Their efficacy varies with the method and severity of the treatments, heat transfer parameters of the matrix, and also the intrinsic heat resistance of the different groups of microorganisms present, notably spore formers (Jay, 1992; Nesvadba, 2014). Since pre-treatment in boiling water also facilitates drying while reducing microbial load, this method is preferred over the puncturing method.

As with other product of animal origin, insects carry various pathogens and high bacterial counts can be expected. As a comparison, minced meat and seafood regulations have formed the basis of the primary microbial risk assessment associated with edible insects due to the lack of better available knowledge (Grabowski and Klein, 2017). Future edible insect guidelines and regulations are expected to require the absence of *Salmonella* and may also recommend levels of TAM, *Staphylococcus aureus* and *E. coli* to be below 10^6, 10^3 and 10 cfu/g, respectively (MAPAQ, 2019). In our study, even after pre-treating larvae in boiling water and then drying, controlling *E. coli* numbers would require further interventions, whereas the standards for TAM were met (Tables 6 and 7). In our study, *Enterobacteriacea* counts remained at 5.30±0.13 log cfu/g even after 8 min of boiling water (Table 2) whereas Larouche *et al.* (2019) were able to reduce their count to below detection level with blanching 40 s. Surprisingly, Zhen *et al.* (2020), using also blanching 40 s, obtained high total viable counts (9.89 log cfu/g) for BSFL raised on fattening broiler chicken diet and palm kernel meal. In other insects, Klunder *et al.* (2012) were able to efficiently reduce *Enterobacteriacea* to less than 1 log cfu/g by boiling crickets and mealworm larvae in water for a few minutes. Vandeweyer *et al.* (2017) also observed several microbial log reductions by blanching mealworm larvae for 40 s followed by microwave drying. Their treatments represented a level of treatment akin to pasteurisation, with little effect on bacterial spores. This suggests that some insects may be more difficult to decontaminate due to their structure, composition and type, as well as level of contamination prior to processing. Fat content is known to reduce heat treatment efficacy (Nesvadba, 2014). High hydrostatic pressure has also been used successfully to reduce microorganisms in BSFL, including *E. coli* O157:H7 (Kashiri *et al.*, 2018).

BSFL gut microbiota may vary to a certain degree depending on diet (Bruno *et al.,* 2019; Klammsteiner *et al.*, 2020; Tanga *et al.,* 2021; Wynants *et al.,* 2019), and the feed and conditions in which the BSFL are raised are likely to affect the efficacy of the processing procedures for microbial risk mitigation. The initial microbial load of larvae will undoubtedly affect the efficacy of antimicrobial treatments and it is therefore best to keep these numbers as low as possible. In addition, the pH of larvae went from 6.5±0.1 in the raw-thawed state (Table 1) to a pH>8 after pre-treatment in boiling water (Table 1) which decreased after drying (7.4-7; Table 8). These results are in agreement with Larouche *et al.* (2019) where BSF larvae pH is reported at 8.7±0.1 and 7.8±0.5 after treatment in boiling water and drying, respectively. However, Zhen *et al.* (2020) obtained a pH below 8 after blanching treatment of 40 s suggesting that many factors, which may include raising conditions and diet, influence the reactivity of the larvae matrix. The high pH (>8.5) after pre-treatment in boiling water are comparable to the alkaline pH of the albumin (8.0) in freshly-laid chicken eggs, which is known to inhibit microbial growth compared to the yolk, which as a more neutral pH (Heath, 1977). As the pH becomes less neutral and more acidic or alkaline, the conditions become less favourable for microbial growth, as is the case for *E. coli* at high pH levels (Geveke, 2008). The increase in pH observed in BSFL during our study may be due to unfolding imidazolium that leads to histidine basic residue exposure during heating, or a decarboxylation reaction, as is observed in other biological systems (Choi *et al.*, 2009; Marshall and Bal'a, 2001). Further investigation is required to accurately identify the mechanisms that are involved in the pH increase that occurs after heat treatment with boiling water.

Product quality

Insects have a high protein content (13-77% of DM) that varies among insect orders and species, depending on the stage of metamorphosis and feeding substrate (Cammack and Tomberlin, 2017; Cappelloza *et al.*, 2019; Nguyen *et al.*, 2013; Valeras, 2019; Van Huis *et al.*, 2013). In this study, the protein content of the dried larvae varied from 35% to almost 40% on a dry basis depending on the larvae pre-treatments prior to hot-air and freeze-drying (Tables 4 and 5). Our results are higher than those reported in the review by Nowak *et al.* (2016), where the average protein concentration (grasshoppers, crickets, flies, butterflies, etc.) was reported to be 17.85 g/100 g of insects which represents 21% of DM. However, the results for protein content in this study are similar to those reported by Huang *et al.* (2019) in their study on BSFL (42.0% of crude protein in dry basis). The differences in protein content for various scalding times, as shown in Table 4, could be related to the loss of soluble proteins into the boiling water. Indeed, Ursu *et al.* (2016) showed that the protein content of ham decreased when boiled. In addition, Purschke *et al.* (2018)

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demonstrated a decrease in the protein content of larvae that were blanched prior to drying (65.5% for oven drying, and 55% for freeze-drying). Low lipid content values were detected for punctured larvae (Tables 4 and 5). This may be due to lipids that were lost by the contents of the larvae dripping out during subsequent steps.

Puncturing increases oxygen accessibility to constituents and therefore can cause colour change notably by enzymatic or non-enzymatic browning reactions (Zhen *et al.*, 2020) and by conversion of ferrous iron present in BSFL into ferric iron which will react with other constituents (Janssen *et al.*, 2018). Boiling as a pre-treatment prior to air-drying allows for oxidation reduction in dried larvae, compared to the control (Figure 3A). This can be explained by enzyme inactivation, but also by the reduction in drying time (Table 3). Moreover, TBARS levels in air-dried larvae increased significantly with the length of scalding time during pre-treatment (Figure 3A). This may be due to the steady removal of the larval cuticle's wax covering, which acts to protect the larvae against oxidation and other deteriorative activities, as a function of scalding time. Thus, even though longer scalding times might reduce hot-air drying processing times and microbial load, it could also decrease lipid quality in dried larvae through oxidation.

After hot air-drying, dried larvae with no pre-treatment showed the largest colour change (Figure 3B) as well as the largest oxidation levels (Figure 3A). Scalding prior to air-drying helped to minimise colour changes relative to the control dried larvae. This was true for all scalding times with no significant differences between them. The colour changes that were observed could also be explained by larvae light reflection, which is higher in fresh larvae, because of their limited absorption capacity due to the high content of extra cellular water (Faustman *et al.*, 1989).

Of all the treatments examined, freeze-drying resulted in the smallest amount of colour change (with or without pre-treatment), reinforcing the connection between oxidation, colour change and drying time. These results are consistent with those from Purschke *et al.* (2018), which showed that freeze-dried blanched larvae maintained their original colour, while those that were oven-dried at 80 °C (blanched) exhibited a marked colour change.

Increases in primary and secondary oxidation indicate that other strategies, such as packaging and processing under vacuum should be considered in order to limit lipid degradation and prevent rancidity.

Storage

For the storage assay, the ground powder from thawed larvae that were scalded for 4 min and then dried with hot air (60 °C; 6 h) was used because hot-air drying was more effective than freeze-drying (Table 3). Although the microbial load was significantly lower among larvae that were scalded for 6 and 8 min, the log reduction was less than one compared to those that were scalded for 4 min (Table 6). Therefore, there was no need to apply a more severe heat treatment that would negatively affect other parameters, such as lipid oxidation (Table 4). The larvae powder remained relatively stable over the 30-day storage period. When properly closed, WhirlPak bags can limit interaction with air and could contribute to storage stability. Water activity did not vary significantly over time and colour changes were limited. Water activity was between 0.24 and 0.37 (Tables 4 and 5), which is considered effective for the control of microbial growth since it is below 0.6 (Rahman, 2007). Indeed, over the 30-d storage period, microbial counts decreased slightly (less than 1 log reduction) or remained stable (yeasts and moulds; Table 9). Furthermore, water activity was also within the 0.2 to 0.4 range for reduced enzymatic reactions and browning (Rahman, 2007). Lipid oxidation rates were also low (Table 8). However, to meet market demand for a long shelf life for dried products, the experiment will need to be repeated over a longer period of time.

The absence of any detectable *Salmonella* is an absolute requirement for both animal feed and human food ingredients (CFIA, 2018). This requirement was met with the larvae powder that was used in the storage experiment. Furthermore, the heat treatments during pre-treatment and drying reduced larval *E. coli* counts by more than 4-log unit for larvae scalded 4, 6 or 8 min (Table 6). One major difficulty, with respect to microbial safety, is the fact that the larvae are raised in close contact with their faecal dejections (Larouche, 2019).

5. Conclusion

Despite some challenges, BSFL farming and processing could help provide the animal feed industry with novel, safe and sustainable protein sources. In this study, larvae pre-treatments in boiling water were deemed important as they are effective in reducing the microbial load, drying time and oxidation in the final dry BSFL product. In terms of drying efficacy, hot-air drying should be considered the preferred method, although the final product turns an intense brown colour. This change in colour is not expected to impact its acceptability as feed ingredient. The water activity achieved in the final product (<0.4) was sufficient to provide product stability and, although additional antimicrobial interventions could further improve the final microbial load, the absence of *Salmonella* suggests that the dried BSFL produced would be fit for use in feed under the current regulations in Canada.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/JIFF2021.0002.

Figure S1. Device used to puncture larvae.

Table S1. The *P*-values for pre-treatments (P), drying (D) and their interactions on the physico-chemical properties of black soldier fly larvae.

Table S2. The *P*-values for pre-treatments (P), drying (D) and their interactions on the microbial load in black soldier fly larvae.

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