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Effect of blanching, storage and drying conditions on the macro-composition, color and safety of mealworm *Tenebrio molitor* larvae

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ABSTRACT

For food applications, the processing conditions applied to edible insects should present low energy requirements and environmental impact, while also assuring high quality and safety. The main goal of this study was to assess how different blanching, storage and drying conditions influence the quality and safety of *Tenebrio molitor* larvae. The different operations included blanching treatments (water-immersion or steaming), post-blanching storage (freezing or refrigeration) and drying methods (oven, microwave or freeze dryer). To monitor the impact on the quality of *T. molitor* dry matter content, water activity (a_w), color and macro-nutrient composition were evaluated, while microbiological analyses were performed to assess the impacts on quality and safety. Blanching, particularly immersion-blanching, improved the lightness of the larvae, both before and after drying. Both blanching treatments reduced the Total Viable Count (TVC) and Enterobacteriaceae loads. Regarding the drying methods, freeze drying led to the best color conservation, while drying with an oven led to the lowest moisture content and a_w. The application of drying methods further contributed to the reduction of TVC and Enterobacteriaceae, assuring low loads even after 3 months of storage, with the freeze dryer resulting in the lowest reductions, including an increase in yeast and mold count.

1. Introduction

Edible insects are traditionally consumed in several regions of the world (mainly in Central/South America, Africa and Southeast Asia), due to their nutritional value and sensory properties (Abril, Pinzón, Hernández-Carrión, & Sánchez-Camargo, 2022; Raheem et al., 2019). In the West, the popularity of entomophagy (intentional consumption of insects) has been growing in the past decade (Baiano, 2020; van Huis, 2022), because insects are seen as a sustainable alternative to current protein sources, due to their nutritional requirements and environmental sustainable production (Ojha, Bekhit, Grune, & Schlüter, 2021; Rumpold & Schlüter, 2013; van Huis & Oonincx, 2017). The European Union has already listed several edible insect species as safe for human consumption, including the yellow mealworm, *Tenebrio molitor* larvae (Turck et al., 2023; Turck et al., 2021a; Turck et al., 2021b), which is one of the most popular species for human consumption in the West (Baiano, 2020; van Huis, 2022). Currently, *T. molitor* has been approved as safe to consume by the European Food Safety Authority in the following forms: frozen (Turck et al., 2021a), freeze-dried (Turck et al., 2021a), oven-dried (Turck et al., 2021b) and oven-dried with UV-treatment (Turck et al., 2023).

In regions where entomophagy is a traditional practice, insects are commonly harvested in the wild and consumed at a local level, while in Western countries there is a mass-production of edible insects and derived products. Therefore, product safety throughout all the processes of rearing and subsequent transformation are critical issues, which have been one of the most extensively studied topics regarding the utilization of insects as a food source (Imathiu, 2020; van der Fels-Klerx, Camenzuli, Belluco, Meijer, & Ricci, 2018). With adequate control of the insects' feed, most risks associated with consumption of insects can be adequately managed, but both allergenicity (Ribeiro, Cunha, Sousa-Pinto, & Fonseca, 2018; Ribeiro, Sousa-Pinto, Fonseca, Fonseca,

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& Cunha, 2021) and microbiological safety should also be taken into account. Although fresh insects rarely present bacterial pathogens (Grabowski & Klein, 2017; Vandeweyer, Crauwels, Lievens, & Van Campenhout, 2017; Vandeweyer et al., 2018; Wynants et al., 2018), they generally present high microbiological load (Garofalo et al., 2019). As such, the processing of edible insects should have a special focus on microbial load, to ensure safety of the final product (Aguilar-Toalá, Cruz-Monterrosa, & Liceaga, 2022; Gałęcki, Bakuła, & Gołaszewski, 2023).

While there are different steps involved in the processing of insects, the most common procedures include starvation, killing by freezing, thermal blanching, drying and grinding (El Hajj et al., 2022; Hernández-Álvarez, Mondor, Piña-Domínguez, Sánchez-Velázquez, & Melgar Lalanne, 2021; Melgar-Lalanne, Hernández-Álvarez, & Salinas-Castro, 2019; van der Fels-Klerx et al., 2018). Starvation is usually applied to empty the gut content and thus theoretically reduce the microbiological load, although some studies have revealed that this step does not have a significant effect on safety (Wynants et al., 2017; Wynants et al., 2018). Thermal blanching of edible insects is almost exclusively performed through immersion in boiling water, and it has been reported to reduce microbiological load and to inactivate enzymes such as phenol oxidase (Hernández-Álvarez et al., 2021; Melgar-Lalanne et al., 2019). Drying is also applied throughout processing, not only to increase shelf-life but also to facilitate the incorporation into food products more easily accepted by consumers. Traditionally, insects are dried through sun-drying, roasting or frying, but in the West other drying methods (freeze dryer and oven) are more commonly applied (Hernández-Álvarez et al., 2021; Melgar-Lalanne et al., 2019). To increase the economic viability of the edible insects' industry, drying methods with lower energy input and higher yield such as microwave-drying (Lenaerts, Van Der Borght, Callens, & Van Campenhout, 2018), radio frequency drying (Vandeweyer et al., 2022) or fluidized bed drying (Kröncke, Böschen, Woyzichovski, Demtröder, & Benning, 2018; Purschke, Brüggen, Scheibelberger, & Jäger, 2018) have also been studied in recent years. From a food quality and safety perspective, it is important to ascertain the water activity (a_w) of the dried products, since a_w below 0.6 inhibits most microbial growth, while enzymatic activity and non-enzymatic browning reactions are reduced at a_w between 0.20 and 0.40. Lipid oxidation is also reduced until a_w of 0.30, but further reductions can lead to the opposite effect (Kröncke et al., 2018; Lenaerts et al., 2018). Furthermore, the applied processing conditions can have an impact on the nutritional content and protein functionality (such as solubility or digestibility) (Meshulam-Pascoviche, David-Birman, Refael, & Lesmes, 2022).

Besides the effects of insect processing on their safety and nutritional content, it is also essential to assess how it can affect the sensory properties of the insect-based products, Furthermore, when deciding processing methods and conditions the initial material and the desired end product should be taken into consideration (Ojha, Bußler, Psarianos, Rossi, & Schlüter, 2021). It has been shown that the incorporation of insects often lead to lower hedonic evaluations, as well as association with negative sensory properties mostly related to flavor/taste, odor and darkening/browning of the products (Cunha & Ribeiro, 2019). The impact of different processing technologies has not yet been thoroughly evaluated, but recent studies have reported that the cooking methods (Seo, Kim, & Cho, 2020), drying methods (Mishyna, Haber, Benjamin, Martinez, & Chen, 2020; Ribeiro, Santos, Lima, Pintado, & Cunha, 2022) or grinding conditions (Bassett, Dunn, Pike, & Jefferies, 2021) can impact the acceptance and sensory properties of insect-based products. Thus, the applied technologies should aim to assure the oxidative stability of the fat fraction, as several studies have pointed out how the fat fraction of edible insects is associated with off-flavors and off-odors (Delicato, Schouteten, Dewettinck, Gellynck, & Tzompa-Sosa, 2020; Ribeiro et al., 2019; Ribeiro et al., 2022; Tzompa-Sosa, Dewettinck, Gellynck, & Schouteten, 2021), but also mitigate the darkening/-browning phenomenon caused by chemical reactions triggered by enzymatic activity of phenol oxidase (Janssen et al., 2017) or through non-enzymatic related activities (melanisation or Maillard reaction) (Kröncke et al., 2019; Leni, Caligiani, & Sforza, 2019).

The present study focused on the effects of processing conditions on the characteristics of edible insects, being a complement to other previous studies (Ribeiro et al., 2022; Santos, Ribeiro, & Cunha, 2021) which assessed the effects of different immersion-blanching and drying conditions on the color and sensory properties of edible insects. More specifically, in this study, different processing conditions related to blanching, storage and drying were applied to *T. molitor* larvae, and their effects on several parameters (color, water activity, macronutrient composition and microbiological content) were evaluated, to determine which processing conditions can lead to optimal *T. molitor* quality and safety. Furthermore, with this study it was also sought to apply processing methods that allow for lower energy input and higher environmental sustainability. Reflecting this, two blanching methods (immersion and steam) were applied, as steam-blanching assures lower water consumption and higher energy efficiency (Fellows, 2009). For drying methods, since freeze-drying is a method that requires extensive energy consumption, more energy-efficient methods (oven and microwave) were also applied (El Hajj et al., 2022; Hernández-Álvarez et al., 2021; Keil et al., 2022). Lastly, considering the high energy consumption associated with freezing, refrigeration was also tested as a method of *T. molitor* storage (Powell-Palm & Rubinsky, 2019).

2. Materials and methods

2.1. Insect samples and experimental design

Yellow mealworm *Tenebrio molitor* larvae obtained from GreenMeal (insect rearing company in Matosinhos, Portugal), were fed organic vegetable waste, and harvested with 9 weeks old. After harvesting, insects were starved for 48 h to eliminate the gut content before being euthanized by freezing at −24 °C. After being euthanized, *T. molitor* were collected by the research team, divided in different samples (*ca.* 400 g) and subjected to different processing conditions (Fig. 1), which were not performed as independent replicates.

The frozen larvae were stored at −24 °C for 24 h (in polyethylene zip lock bags) before being blanched under different conditions (immersion or steam). Immersion-blanching (IB) was performed according to Santos et al. (2021). The larvae were immersed in water at 100 °C (heat supplied by heating plate) for 5 min in a ratio of 1:10 (w/v; 100 g to 1 L of water). After blanching, the water and larvae were poured onto a stainless-steel sieve, leaving the blanched larvae, which were transferred into previously identified polyethylene zip lock bags. Steam-blanching (SB) was carried out at ambient pressure in a steam cooker machine (Tefal® model VS400333 VitaCuisine Compact). Before the placement of the larvae in the basket (*ca.* 250 g), the steam cooker was used for 1 min to warm the water before blanching. Steam-blanching was performed for 5 min, after which larvae were transferred into previously identified polyethylene zip lock bags.

Immediately after blanching, the samples were stored under two different conditions – frozen at −24 °C, or refrigerated at 4 °C, for 24 h, before the application of drying conditions. After the post-blanching storage, larvae treated with four different conditions were obtained: immersion-blanched and frozen (IB–F), immersion-blanched and refrigerated (IB-R), steam-blanched and frozen (SB–F) and steam-blanched and refrigerated (SB-R).

Three different drying methodologies were investigated to assess their effect on quality and safety parameters of *T. molitor* larvae: electrical oven (O), microwave (Mw) and freeze dryer (FD). The conditions for each drying method were defined considering current targets of moisture content (<5.0 g/100 g) and a_w (<0.6), present on *T. molitor* processes approved by the EFSA (Turck et al., 2023; Turck et al., 2021a; Turck et al., 2021b), and considering further incorporation into food products (e.g., drying conditions could not lead to burnt larvae).

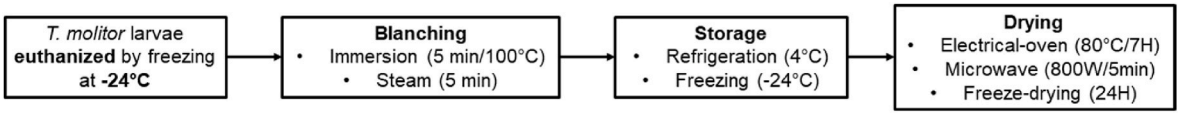

Fig. 1. Flow chart and overview of the 10 applied processing methodologies (for freeze-drying, only samples with post-blanching storage in a freezer were used).

Electrical oven drying was performed in a conventional oven (Unox® model XF016-TG) at 80 °C for 7 h (Ribeiro et al., 2022; Santos et al., 2021), with 1 kg of larvae being divided into two trays. Microwave-drying was performed in a conventional microwave (Teka® MW 21 IVS INOX), with the larvae (*ca.* 50 g) being distributed in a ceramic plate and dried at a power level of 800 W, for 5 min (Ribeiro et al., 2022; Santos et al., 2021). For freeze-drying (Telstar® LyoQuest −55, coupled with vacuum pump Ulvac® model GLD136C), only frozen larvae stored after blanching (IB–F and SB-F) were used. Larvae (*ca.* 250 g) were subdivided into two freeze-drying plates, and freeze-drying was applied with the following conditions: freezing for 3 h at −50 °C, followed by freezing with the application of vacuum (0.250 mbar) for 3 h, and 24 h with heated shelves (35 °C, 0.250 mbar). Conditions were chosen based on preliminary studies with varying duration (24 h, 48 h or 72 h). All the dried larvae were ground in a kitchen robot (Kenwood® Major Titanium with the multi-mill attachment model AT320A) for further characterization.

Estimates of energy consumption (EC) of the different blanching and drying methods were calculated according to Keil et al. (2022), with the assumption that the equipment ran at maximum capacity. For oven-drying, EC was calculated according to the following equation (Eq. 1):

$$EC\ (kWh) = \frac{D_T}{M_T} \times MO \times t \quad \text{(Eq. 1)}$$

where D_T is the drying temperature (°C), M_T is the maximum drying temperature (°C), MO the maximum power output (kW) and t is the drying time (h).

For the other equipment, EC was calculated according to the following equation (Eq. 2)

$$EC\ (kWh) = MO \times t \quad \text{(Eq. 2)}$$

Results were converted to estimate the energy consumption required to either blanch or dry 1 kg of *T. molitor* (Table S1). For the blanching methods there were small differences in estimated energy consumption, although immersion-blanching led to higher values (1.2 kWh/kg) (Table S1). Furthermore, to immersion-blanch 1 kg of *T. molitor* larvae there was a greater water consumption (*ca.* 3 L) than for steam-blanch (*ca.* 1 L). On the other hand, for the drying methods there were major differences since the estimated energy consumption of the freeze dryer (300 kWh/kg) was much greater than the estimated energy consumption of either the oven (4.85 kWh/kg) or the microwave (1.28 kWh/kg) (Table S1).

2.2. Quality, nutritional and safety characterization

2.2.1. Dry matter

Dry Matter (DM) content was determined on both dried and non-dried samples. For non-dried samples, DM was determined prior to blanching, while for blanched samples after frozen or refrigerated storage. For the non-dried samples, the analysis was performed with the whole larvae. All measurements were performed in triplicate and with the assistance of an analytical balance (Ohaus Explorer® model E01140).

To determine the DM content of the samples, petri dishes were identified and weighed (W1), and the samples were then weighed (W2). Subsequently, the petri dishes with the samples were placed in an air-flow lab incubator (Binder® APT. line series model ED115) at 95 °C, for 24 h, and placed in a desiccator until room temperature was reached. Afterwards, the petri dishes with the dried samples were weighed (W3). DM content was calculated with the following equation (Eq. (3)):

$$DM\ (g/100\ g) = \frac{W3 - W1}{W2} \times 100 \quad \text{(Eq. 3)}$$

2.2.2. Water activity

Water activity (a_w) was calculated as the mean of triplicate measurements for all the dried samples (Rotronic® HygroLab 3).

2.2.3. Color

For color evaluation, the CIELAB color space ($L^*a^*b^*$) was used, with measurements being performed with a colorimeter (Minolta® Chroma Meter model CR-400), using illuminant D65, to better replicate daylight. L^* is the lightness value (that defines black as 0 and white as 100), the a^* axis corresponds to the green–red colors (where negative values correspond to green and positive ones to red) and the b^* axis corresponds to blue-yellow colors (where negative values correspond to blue and positive ones to yellow).

Color was measured for both non-dried and dried samples. For non-dried samples, color was measured for whole larvae in three different moments: prior to blanching, after blanching and after storage (before drying). For all the samples, color was evaluated as a mean of six replicates.

Total color differences (ΔE) were also calculated according to Eq. (4):

$$\Delta E = \sqrt{\left(L^*_{ref} - L^*_{sample}\right)^2 + \left(a^*_{ref} - a^*_{sample}\right)^2 \left(b^*_{ref} - b^*_{sample}\right)^2} \quad \text{(Eq. 4)}$$

The color coordinates of the reference samples (L^*_{ref}, a^*_{ref}, b^*_{ref}) were different according to the assessed process: for blanching, the color coordinates of the reference sample were those of non-blanched samples; for post-blanching storage, the color coordinates of the reference sample were those of samples blanched prior to storage.

2.2.4. Macro-nutrient composition

The macro-nutrient composition of both non-dried (non-blanched and blanched) and dried samples was analyzed. However, only the samples that went through frozen (−24 °C) post-blanching storage were considered for this analysis. Crude protein content was determined according to the Kjeldahl method with a Nitrogen conversion factor of 6.25. Briefly, 0.2 ± 0.01 g of sample and 1 g ± 0.01 g of Kjeldahl catalyst were weighed into a Kjeldahl digestion tube, and then 4 mL oh H_2SO_4 96% was added to each tube, which were then digested in a Kjeldahl digestion system at 416 °C until a clear green color was observed. After digestion, 20 mL of distilled water was added to each tube, and samples were distilled and titrated with a 0.1 mol/L HCl solution. Crude fat content was determined with the Soxhlet method with petroleum ether as a solvent. Samples (2.5 ± 0.2 g) were initially hydrolyzed with a 3 mol/L HCl solution for 1 h at boiling temperature. Samples were then filtrated, washed until pH neutrality, and dried. The filters with the dried samples were transferred into cartridges and extraction was performed with 140 mL of petroleum ether on a semi-automatic Soxhlet apparatus. Crude ash content was determined gravimetrically after incineration of the samples (2.5 ± 0.2 g) in a muffle furnace at 550 °C, for 3 h. All the determinations were performed in duplicate.

2.2.5. Microbiological analysis

For the microbiological analyses, only samples having post-

blanching storage in a freezer (−24 °C) were considered, and a similar approach to previously described in section 2.3.4 was applied. Furthermore, all analyses were performed both after blanching (non-dried samples) or drying, and three months after storage. Non-dried samples were stored in a freezer inside polyethylene zip lock bags, while dried samples were stored inside air-tight glass flasks at room temperature. Additionally, the non-dried samples were ground before evaluating their microbiological load.

The microbiological content (Total Viable Count, Enterobacteriaceae, Bacterial Endospores, Yeasts & Molds) was determined according to Vandeweyer, Crauwels, et al. (2017) and Vandeweyer, Lenaerts, Callens, and Van Campenhout (2017) with slight modifications. Ground samples (5 g) were initially homogenized with 45 mL of buffered peptone water (Scharlab, Barcelona, Spain). After homogenization, tenfold dilution series were prepared and plated on different agar media (Liofilchem, Roseto degli Abruzzi (TE), Italy) using the pour-plate technique. Total Viable Counts (TVC) were determined on Plate Count Agar (PCA) after incubation for 72 h at 30 °C, Enterobacteriaceae on Violet Red Bile Glucose Agar for 24 h at 37 °C, and Yeasts & Molds on spread plates of Dichloran Rose-Bengal Chloramphenicol Agar supplemented with Chloramphenicol after incubation for 5 days at 25 °C. Aerobic bacterial endospores were determined after a pasteurization treatment of 10^{-1} dilution at 80 °C for 10 min, followed by dilution and incubation on PCA for 48 h at 37 °C. All determinations were performed in triplicate.

2.3. Statistical analysis

All data was analyzed using the software Statistical Package for Social Sciences (SPSS) - version 27 ® with all the statistical tests being applied at 95% confidence level. All the results are presented with descriptive statistics (frequencies, mean and standard deviation).

Color of non-dried samples was evaluated immediately after blanching and after post-blanching storage, using a One-Way ANOVA, with either blanching method or post-blanching storage condition as factors.

Dry matter, water activity and color of dried samples were initially evaluated with a Two-Way ANOVA, with blanching method and post-blanching storage condition as fixed factors. Then, for the comparison between drying methods, the results obtained from blanched and frozen (−24 °C) samples were analyzed with a Two-Way ANOVA, with drying and blanching methods as fixed factors.

For the analysis of the macro-nutrient composition, non-dried samples were evaluated with a One-Way ANOVA with blanching method as a factor. For the evaluation of dried samples, a Two-Way ANOVA was performed, with drying method and blanching conditions as fixed factors.

For the analysis of the microbiological content results, within each storage time (T0 and T3), the effects of blanching on the microbiological content of both dried and non-dried samples, a One-Way ANOVA was applied with the blanching method as a factor. For evaluation of drying methods (non-dried, oven, microwave, freeze dryer), a One-Way ANOVA was applied with the drying method as a factor. Then, the effect of storage time was evaluated with a One-Way ANOVA with storage time as a factor.

When applicable, *post-hoc* analysis was applied with the Tukey's test for comparison between multiple groups.

3. Results and discussion

3.1. Effects of blanching and post-blanching storage

3.1.1. Non-dried samples

Prior to drying, both blanching treatment and post-blanching storage had significant effects on the color of the samples (Table 1). Immersion-blanching significantly increased the lightness values L^* (50.2 ± 1.4) when compared to the non-blanched samples (46.5 ± 1.3). On the other hand, a^* (5.3 ± 0.2) and b^* (26.2 ± 0.8) values were significantly decreased in comparison to non-blanched samples (9.0 ± 1.2 and 29.6 ± 2.1, respectively). Steam-blanching caused similar effects as immersion-blanching, but significant effects were only observed for a^* values. Reflecting these differences, immersion-blanching led to significantly higher ΔE than steam-blanching. Nevertheless, since both blanching treatments caused $\Delta E > 3.0$, color modifications can be classified as very distinct (Purschke et al., 2018). Regarding the effects of post-blanching storage, freezing the samples after blanching led to higher L^* values but lower a^* values (although with only very small differences) while it did not affect the b^* values. Frozen samples maintained a higher color integrity through the duration of storage, as they presented significantly lower ΔE. These results support the idea that until insects are dried, they should be kept frozen, to maintain their color properties. In fact, in preliminary experiments performed in this study, defrosting the samples before blanching treatments led to worse effects in the color of samples throughout all the processing stages (Figs. S1 and S2).

Table 1
CIE*LAB* color coordinates and ΔE values (Mean (±S.D), n = 6) of non-dried *T. molitor* according to either blanching treatment or post-blanching storage condition. a,b,c – homogenous groups in each column according to Tukey's *post hoc* test ($p < 0.05$). #significantly higher results according to One-way ANOVA.

Blanching	L^*	a^*	b^*	ΔE
Non-blanched	46.5 (±1.3)[b]	9.0 (±1.2)[a]	29.6 (±2.1)[a]	—
Immersion	50.2 (±1.4)[a]	5.3 (±0.2)[c]	26.2 (±0.8)[b]	6.3 (±1.0)[#]
Steam	48.5 (±2.0)[ab]	6.8 (±0.3)[b]	27.5 (±1.9)[ab]	4.3 (±1.1)
Post-blanching storage	L^*	a^*	b^*	ΔE
Frozen	49.3 (±1.9)*	6.1 (±0.8)	26.9 (±1.5)	1.8 (±1.0)
Refrigerated	41.3 (±3.2)	6.8 (±0.9)*	26.4 (±2.7)	8.7 (±3.0)[#]

This positive effect of blanching on the color of edible insects has been reported in other studies with *T. molitor* (Cacchiarelli et al., 2022; Mancini et al., 2019; Santos et al., 2021) or *H. illucens* (Larouche et al., 2019; Leni et al., 2019). These positive effects can be caused due to inactivation of phenol oxidase (which occurs at high temperatures), which can be responsible for browning verified on insect products (Janssen et al., 2017). Furthermore, in most cases, blanching is used as a treatment following euthanasia, though Leni et al. (2019) reported that using blanching as a killing method presented advantages over other common euthanasia methods. Namely, a slow killing method by freezing elicits the activation of several enzymatic pathways, among them melanisation. Conversely, blanching can inactivate phenol oxidase (inhibiting browning reactions), avoiding the activation of metabolic pathways induced by the stress associated with slow killing by freezing.

However, there have also been studies that reported a negative effect of blanching on the color of *T. molitor* (Lenaerts et al., 2018) or *H. illucens* (Saucier et al., 2022). However, it is important to note that Lenaerts et al. (2018) evaluated the color of fresh and blanched samples on different batches. Interestingly, Saucier et al. (2022) evaluated both blanching (40 s) and scalding, and reported that although both methods decreased L^* and b^* values, these effects were more pronounced for scalding up to 8 min than for blanching (40 s).

3.1.2. Dried samples

Some of the effects that blanching treatment and post-blanching storage had for non-dried samples, were still maintained after drying the samples (Table 2). For all drying methods, the immersion-blanched samples, and samples with frozen post-blanching storage, presented

Table 2
CIE*LAB* color coordinates (Mean (±S.D), n = 6), dry matter (g/100 g) and water activity (a_w) content (Mean (±S.D), n = 3) of dried *T. molitor* according to either blanching treatment or post-blanching storage condition. #Significantly higher results for CIE*LAB* color coordinates according to Two-Way ANOVA. *Significantly higher results for either dry matter (g/100 g) or a_w content according to Two-Way ANOVA.

Oven					
Blanching	L^*	a^*	b^*	Dry matter (g/100 g)	a_w
Immersion	39.5 (±1.9)#	10.1 (±0.3)	25.9 (±0.7)	99.9 (±0.7)*	0.078 (±0.006)
Steam	35.6 (±2.1)	12.1 (±0.2)#	25.7 (±1.6)	99.0 (±0.0)	0.135 (±0.009)*
Post-blanching storage	**L^***	**a^***	**b^***	**Dry matter (g/100 g)**	**a_w**
Frozen	39.4 (±2.0)#	11.0 (±1.2)	26.7 (±0.6)#	99.2 (±0.4)	0.107 (±0.038)
Refrigerated	35.7 (±2.2)	11.2 (±0.9)#	24.9 (±0.9)	99.7 (±0.8)	0.106 (±0.026)
Microwave					
Blanching	L^*	a^*	b^*	Dry matter (g/100 g)	a_w
Immersion	38.6 (±2.0)#	13.7 (±0.7)	28.3 (±0.8)#	98.1 (±1.0)	0.386 (±0.068)*
Steam	35.8 (±1.7)	13.9 (±0.5)	26.7 (±1.1)	97.1 (±1.2)	0.223 (±0.032)
Post-blanching storage	**L^***	**a^***	**b^***	**Dry matter (g/100 g)**	**a_w**
Frozen	38.8 (±1.8)#	13.6 (±0.7)	27.8 (±1.5)	97.0 (±1.1)	0.312 (±0.135)
Refrigerated	35.7 (±1.5)	14.0 (±0.4)#	27.2 (±0.9)	98.2 (±0.9)	0.294 (±0.050)
Freeze dryer					
Blanching	L^*	a^*	b^*	Dry matter (g/100 g)	a_w
Immersion	46.3 (±2.3)#	8.8 (±0.3)	24.2 (±0.5)	97.9 (±1.8)*	0.036 (±0.001)
Steam	41.9 (±1.0)	10.3 (±0.2)#	26.5 (±0.2)#	93.2 (±0.7)	0.406 (±0.001)*

higher L^* values. The effect of blanching is particularly interesting, since there were no significant differences between blanching methods prior to drying (Table 1). Another common effect is higher a^* values for steam-blanched samples and samples from refrigerated post-blanching storage. Furthermore, some effects on the b^* values were also observed. As it occurred for the samples prior to drying (Table 1), immersion-blanching led to higher values but only for microwave-dried samples. On the other hand, opposite results were verified for freeze-dried samples, but these effects could have occurred due to a decreased drying of these samples (Table 2). Furthermore, all the drying methods led to decreases in L^* values and slight increases in the a^* values, compared to the non-dried samples.

Formerly, several studies have reported that prior blanching can have a positive effect of L^* and b^* values of edible insects dried with different methods: hot-air (Azzollini, Derossi, & Severini, 2016; Saucier et al., 2022), oven (Lee et al., 2023; Purschke et al., 2018), vacuum (Seho et al., 2022) and freeze dryer (Saucier et al., 2022). Concerning the comparison between immersion and steam-blanching, Lee et al. (2023) reported very small differences between oven-dried *H. illucens* blanched with either immersion or steam, with immersion leading to slightly higher b^* values. On the other hand, Saucier et al. (2022) reported that immersion-blanching was more effective for hot-air drying, while scalding was more effective for freeze-drying.

Regarding the effects of blanching treatment methods or post-blanching storage conditions on the dry matter content and a_w of dried samples, only the blanching treatment had an effect (Table 2). Immersion-blanched samples presented higher dry matter content for all the drying methods, but differences were very small for both oven and microwave. For samples dried with a freeze dryer, steam-blanching led to much worse parameters, with a lower dry matter content (93.2 ± 0.7 *vs* 97.9 ± 1.8) and higher a_w (0.406 ± 0.001 *vs* 0.036 ± 0.001). Steam-blanching also led to a higher a_w with oven-dried samples (although with small differences) while the opposite occurred for microwave-dried samples. Lower a_w in immersion-blanched samples subjected to drying with a freeze dryer or oven was also reported by Saucier et al. (2022).

3.2. Comparison between drying methods

For the comparisons between drying methods, only the results from blanched and frozen sample were considered for analysis. Overall, oven-dried samples presented better results in terms of dry matter and a_w (Table 3). Microwave-dried samples also presented acceptable dry matter (g/100 g) content (97.0 ± 1.1) but had the worst a_w values. On the other hand, freeze-dried samples had the worst dry matter (g/100 g) results, but as previously seen, this occurred due to the poor drying of steam-blanched samples (Table 2). The steam-blanching treatment led to lower dry matter content as well as higher a_w values. However, these results should be interpreted with caution since these differences arose from the poor drying of steam-blanched samples with the freeze dryer.

Relative to the color of the dried samples (Table 3), freeze drying is the drying method that allows to obtain *T. molitor* larvae with lightest color (as demonstrated by higher L^* values). This drying method also led to the lowest a^*, b^* and ΔE values, while drying with a microwave led to the highest values in all the parameters (although differences for b^* values were very small between drying methods). Furthermore, these analyses further confirmed that immersion-blanching led to higher L^* and lower b^* values, even after the application of the drying methods. Immersion-blanched samples also maintained a higher color integrity (lower ΔE values). The results presented here have also been extensively reported in literature, with several studies observing higher L^* values for freeze-drying, when compared to other drying methods: air-drying (Azzollini et al., 2016), oven (Purschke et al., 2018), microwave (Lenaerts et al., 2018) and rack-oven (Kröncke et al., 2019). The better effects of drying with a freeze dryer, on the color of edible insects can be ascribed to omission of thermal browning reactions (Purschke et al., 2018) and the fact that high temperatures in the oven or microwave favor non-enzymatic browning, leading to the formation of colored Maillard products, most likely responsible for the lower lightness (Kröncke et al., 2019; Lenaerts et al., 2018).

3.3. Macro-nutrient composition

For the analysis of the effect of different processing conditions on the macro-nutrient composition of *T. molitor* larvae, the non-dried and dried samples were analyzed separately.

Concerning the effect of different blanching conditions on the macro-nutrient composition of non-dried samples (Table 4), both blanching methods significantly increased the moisture content and decreased the fat content. Immersion-blanching also diminished ash content, while neither blanching method had a significant effect on protein content. Increases in moisture content after blanching have been previously reported (Azzollini et al., 2016; Purschke et al., 2018; Santos et al., 2021; Saucier et al., 2022; Vandeweyer, Lenaerts, et al., 2017). This effect could be due to the absorption and entrapment of water inside the larva of *T. molitor* just below the chitinous exoskeleton. Furthermore, Saucier et al. (2022) reported higher increases in moisture content with scalding than with immersion, but this only occurred with very different blanching times (4 min for scalding and 40 s for immersion). Despite this increase in moisture content, the application of blanching treatments can improve drying kinetics (meaning that less time is required to

Table 3
Dry matter (g/100 g), water activity (a_w) content (Mean (±S.D), n = 3) and CIE*LAB* color coordinates and *ΔE* values (Mean (±S.D), n = 6) of dried *T. molitor* according to either blanching treatment or drying methods. a,b,c – homogenous groups in each column according to Tukey's *post hoc* test ($p < 0.05$). *Significantly higher results for dry matter (g/100 g) or a_w content according to Two-Way ANOVA. #Significantly higher results for CIE*LAB* color coordinates and *ΔE* values according to Two-Way ANOVA.

Drying method	Dry matter (g/100 g)	a_w	*L**	*a**	*b**	*ΔE*
Oven	99.2 (±0.4)^a	0.107 (±0.038)^c	39.4 (±2.0)^b	11.0 (±1.2)^b	26.7 (±0.6)^b	11.1 (±1.2)^b
Microwave	97.0 (±1.1)^b	0.312 (±0.135)^a	38.8 (±1.8)^b	13.6 (±0.8)^a	27.8 (±1.5)^a	13.2 (±0.7)^a
Freeze dryer	95.6 (±2.7)^c	0.221 (±0.203)^b	44.1 (±2.9)^a	9.5 (±0.8)^c	25.6 (±1.0)^c	6.5 (±1.7)^c
Blanching	**Dry matter (g/100 g)**	**a_w**	***L****	***a****	***b****	***ΔE***
Immersion	98.3 (±1.0)*	0.179 (±0.190)	42.7 (±3.0)#	10.6 (±1.9)	26.7 (±1.8)	9.5 (±1.2)
Steam	96.2 (±2.6)	0.247 (±0.122)*	38.9 (±2.3)	12.2 (±1.7)#	26.8 (±1.0)	11.1 (±2.7)#

Table 4
Nutritional content of non-dried samples according to blanching treatment and dried samples according to either drying methods or blanching treatment (Mean (±S.D), n = 2). a,b,c – homogenous groups in each column according to Tukey's *post hoc* test ($p < 0.05$).

Blanching	Non-dried samples			
	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)
Non-blanched	60.8 (±0.6)^b	17.6 (±1.1)	12.8 (±0.3)^a	1.5 (±0.1)^a
Immersion	67.5 (±1.8)^a	17.3 (±1.1)	11.3 (±0.5)^b	1.1 (±0.0)^b
Steam	65.7 (±1.1)^a	19.1 (±0.3)	11.2 (±0.4)^b	1.4 (±0.1)^a

Blanching	Dried samples			
	Moisture (g/100 g)	Protein (g/100 g DM)	Fat (g/100 g DM)	Ash (g/100 g DM)
Immersion	1.8 (±1.0)^b	49.0 (±0.6)	31.6 (±0.7)^a	3.3 (±0.1)^b
Steam	3.8 (±2.6)^a	48.9 (±1.3)	29.4 (±1.1)^b	3.7 (±0.2)^a

Drying	Dried samples			
	Moisture (g/100 g)	Protein (g/100 g DM)	Fat (g/100 g DM)	Ash (g/100 g DM)
Oven	0.8 (±0.4)^b	49.3 (±0.9)^{ab}	29.9 (±2.0)	3.7 (±0.3)
Microwave	3.1 (±1.1)^{ab}	49.5 (±0.7)^a	31.0 (±1.2)	3.5 (±0.2)
Freeze dryer	4.4 (±2.6)^a	48.1 (±0.7)^b	30.6 (±0.8)	3.4 (±0.1)

achieve the target dry matter values) (Saucier et al., 2022).

Azzollini et al. (2016), Purschke et al. (2018) and Saucier et al. (2022) did not report any differences regarding nutritional content between non-blanched and blanched samples. On the other hand, Nyangena et al. (2020) reported increases in protein content and decreases in ash content of four different species after boiling. Opposite results were reported by Lenaerts et al. (2018), with blanching leading to small but significantly lower protein content, but this study also reported decreased ash content after blanching. In this study, blanching only caused small decreases in fat and ash content (particularly immersion-blanching), which might have been caused by a leaching effect. Furthermore, major differences might have been related to fiber and carbohydrate content (calculated here as the difference to the sum of moisture, protein, fat and ash content), since it decreases from 7.46 g/100 g in the non-blanched samples to 2.65–2.80 g/100 g in blanched larvae (complex carbohydrates could have dissolved or be washed off the tissues) (Nyangena et al., 2020).

For the dried samples, it was possible to observe an effect of the blanching treatment, with steam-blanched samples presenting lower fat contents and higher ash and moisture contents (Table 4). Saucier et al. (2022) also reported an effect of prior blanching treatments on the nutritional content of dried (hot-air or freeze dryer) *H. illucens*. Prior blanching had contrasting results depending on drying methods, with decreases for hot-air and increases for freeze dryer. Protein content was also decreased with prior blanching for both drying treatments, although effects were greater with immersion blanching. On the other hand, lipid content was only affected in hot-air dying where scalding led to an increase (Saucier et al., 2022). Concerning the effects of different drying methods (Table 4), it only influenced protein and moisture content, with freeze-dried samples presenting the highest and lowest moisture and protein content, respectively.

Fombong, Van Der Borght, and Vanden Broeck (2017), Lenaerts et al. (2018) and Selaledi and Mabelebele (2021) reported no differences in nutritional composition of insects dried with different methods (oven, microwave or freeze dryer), while Kröncke et al. (2018) and Kröncke et al. (2019) also reported lower protein content for freeze-dried insects.

Dried samples also presented lower protein and fat content (on a dry matter basis) than blanched samples, which has also been reported in other studies (Lenaerts et al., 2018). Authors suggest that the decrease in protein content during drying may be related to protein denaturation and/or browning reactions in which part of the amino acids are used.

3.4. Microbiological analysis

Besides the macro-nutrient composition, the microbiological content of *T. molitor* subjected to different processing conditions was also analyzed. The effect of blanching on non-dried samples was initially analyzed (Table 5). Both immersion and steam-blanching led to TVC reduction at both T0 (immersion – −28.8%; steam – −42.4%) and T3 (immersion – −36.1%; steam – −39.8%). Interestingly, steam-blanching was more effective than immersion-blanching at reducing TVC at T0. However, for all samples, a significant increase in TVC was observed between T0 and T3 (non-blanched – 28.9%; immersion – 20.8%; steam – 32.0%). This result was unexpected since a similar study reported no differences in TVC of boiled crickets stored in a freezer up to 6 months (Vandeweyer et al. (2018). Furthermore, all the blanched samples presented TVC below the current recommended thresholds (*ca.* 10^6 CFU/g) (Garofalo et al., 2019). For Enterobacteriaceae load, significant reductions were observed with both immersion and steam treatments, at T0 and T3 (with 84.6% decrease for T0 and 53.1% for T3). The reduction in Enterobacteriaceae was the highest observed for all the analyzed bacteria. This result is in accordance with other studies, since these bacteria are very susceptible to heat treatment (Vandeweyer, Lenaerts, et al., 2017; Wynants et al., 2018). Moreover, Enterobacteriaceae load thresholds have been defined as 10^3 CFU/g (Garofalo et al., 2019), and although blanched samples had counts below this threshold at T0, after 3 months of storage, both blanched samples had Enterobacteriaceae counts very close to this limit (Table 5). Several studies have reported similar results as the ones presented here, in particular for the reduction of TVC and Enterobacteriaceae with immersion-blanching (Cacchiarelli et al., 2022; Mancini et al., 2019; Megido et al., 2017; Saucier et al., 2022; Vandeweyer, Lenaerts, et al., 2017; Wynants et al., 2018) and steam-blanching (Saucier et al., 2022; Stoops et al., 2017)}. However, Vandeweyer, Lenaerts, et al. (2017), Wynants et al. (2018) and Stoops et al. (2017) reported that blanching is ineffective in reducing bacterial spores count, which was also observed in this study. However, in this

Table 5
Microbiological load (log_{10}(CFU/g) (±S.D), n = 3) of non-dried and dried samples according to blanching treatment for each storage time, and non-dried and dried samples according to drying method for each storage time. a,b,c – homogenous groups in each column according to Tukey's *post hoc* test ($p < 0.05$). **Results in bold** symbolize significantly higher results for blanching treatment in the respective storage time.

	Non-dried samples (blanching effect)							
Blanching	T0 (immediately after treatment)				T3 (after 3 months of storage)			
	TVC	*Enterobac-teriaceae*	Endospo-res	Yeasts & Molds	TVC	*Enterobac-teriaceae*	Endospo-res	Yeasts & Molds
Non-blanched	5.9 (±0.8)^{a}	6.5 (±0.0)^{a}	1.7 (±1.5)	1.7 (±2.9)	**8.3 (± 0.1)^{a}**	6.4 (±0.1)^{a}	2.0 (±0.0)	0.0 (±0.0)
Immersion	4.2 (±0.1)^{b}	1.0 (±1.7)^{b}	0.7 (±1.2)	0.0 (±0.0)	**5.3 (± 0.1)^{b}**	3.1 (±0.1)^{b}	0.7 (±1.2)	0.0 (±0.0)
Steam	3.4 (±0.3)^{c}	1.0 (±1.7)^{b}	2.5 (±0.1)	0.0 (±0.0)	**5.0 (± 0.1)^{c}**	2.9 (±0.1)^{b}	0.7 (±1.1)	0.0 (±0.0)
	Dried samples (blanching effect)							
Blanching	T0 (immediately after treatment)				T3 (after 3 months of storage)			
	TVC	*Enterobac-teriaceae*	Endospo-res	Yeasts & Molds	TVC	*Enterobac-teriaceae*	Endospo-res	Yeasts & Molds
Immersion	2.8 (±0.4)	1.2 (±1.4)	0.4 (±0.7)^{b}	1.5 (±2.2)	2.1 (±1.3)	0.8 (±1.3)	0.0 (±0.0)^{b}	1.1 (±1.2)
Steam	2.8 (±0.6)	0.5 (±1.1)	1.7 (±1.3)^{a}	2.4 (±1.9)	2.7 (±1.4)	0.7 (±1.1)	1.5 (±1.2)^{a}	1.6 (±1.9)
	Non-dried and dried samples (drying effect)							
Drying	T0 (immediately after treatment)				T3 (after 3 months of storage)			
	TVC	*Enterobac-teriaceae*	Endospo-res	Yeasts & Molds	TVC	*Enterobac-teriaceae*	Endospo-res	Yeasts & Molds
Non-dried	4.5 (±1.1)^{a}	2.8 (±3.0)^{a}	1.7 (±1.3)	0.6 (±1.7)^{b}	**6.2 (± 1.6)^{a}**	4.1 (±1.7)^{a}	1.1 (±1.1)	0.0 (±0.0)^{b}
Oven	3.1 (±0.2)^{b}	0.0 (±0.0)^{b}	1.5 (±1.6)	0.5 (±1.2)^{b}	2.2 (±1.1)^{c}	0.0 (±0.0)^{c}	1.4 (±1.5)	0.5 (±0.6)^{b}
Microwave	**2.3 (± 0.3)^{b}**	0.4 (±1.1)^{b}	**0.7 (± 0.7)**	1.1 (±1.6)^{b}	1.2 (±0.2)^{c}	0.3 (±0.8)^{bc}	0.0 (±0.0)	0.7 (±1.2)^{b}
Freeze dryer	3.1 (±0.3)^{b}	2.1 (±1.1)^{a}	1.0 (±1.1)	**4.3 (± 0.1)^{a}**	**4.0 (± 0.4)^{b}**	2.0 (±3.0)^{b}	0.9 (±1.0)	2.9 (±1.5)^{a}

study, the load of spore-forming bacteria was low for all samples, at both time-points. A similar situation also occurred for Yeasts & Molds, where blanching did not have any effect, as loads were extremely low (at T3 no CFU were detected).

A comparison between immersion or steam-blanching was only performed by Saucier et al. (2022), where it was demonstrated that steam-blanching provided higher reduction for TVC and Enterobacteriaceae than immersion blanching. However, these effects were only observed for steaming during 6 or 8 min, while a lower steaming duration provided very similar results as blanching for 40 s.

Regarding the effect of blanching treatments on dried samples, only slight differences in microbial load were observed (Table 5). The only significant difference was registered for endospores, where steam-blanched samples had higher counts than immersion-blanched samples, at both T0 and T3.

Lastly, the effect of drying methods was analyzed (oven, microwave or freeze dryer) on microbial load (Table 5). Interestingly, significant differences between non-dried and dried samples, as well as between drying methods were found. For TVC load, a significant reduction was observed at both T0 (ranging between 31.1% for oven and freeze-drying and 48.9% for microwave) and T3 (ranging between 35.4% for freeze-drying and 80.6% for microwave) for all tested methods. Although there were no differences between drying methods at T0; at T3, both microwave and oven-dried samples had lower counts than freeze-dried samples. In fact, freeze-dried samples were the only dried samples with an increase in TVC between T0 and T3, mostly observed for steam-blanched + freeze-dried samples, which presented the lowest dry matter content (93.2%) and a_w values (0.406) among the dried samples (Table 2). The effectiveness of microwave and oven-drying approaches was even reinforced for Enterobacteriaceae counts, since an almost complete reduction at both T0 and T3 was observed. On the other hand, freeze-dried samples presented higher Enterobacteriaceae counts than the other dried samples, at both T0 and T3. As previously described, the reduction of Enterobacteriaceae counts can be mainly explained based on bacterial sensitivity to heat, which might explain the lower effectiveness of the freeze dryer. However, it also must be considered that solely applying blanching can already significantly reduce both TVC and Enterobacteriaceae (Table 5). The application of drying treatments is particularly important, to not only further reduce these loads but to also improve the shelf-life, as dried samples had significantly lower loads than blanched samples at T3 (Table 5).

For endospores counts, there were no differences between either non-dried and dried samples or between drying conditions, at both T0 and T3. However, microwave-dried samples had higher counts at T0 than at T3, but these counts were extremely low. For Yeasts & Molds count, an unexpected result was found, with freeze-dried samples showing higher counts than the other conditions, at both T0 and T3, being significantly higher at T0 when compared with T3.

Vandeweyer, Lenaerts, et al. (2017) reported that drying with a microwave did not lead to further reduction of microbial counts when compared to blanched samples, even when using dried samples with a $a_w < 0.3$. Furthermore, Vandeweyer et al. (2018) reported that oven-dried crickets had increased TVC when compared to boiled crickets. The authors hypothesize that this increase could have been caused by cross-contamination on the equipment or equipment and installations, which can also partially justify the high Yeasts & Molds counts verified for freeze-dried insects in this study. On the other hand, Megido et al. (2017), Saucier et al. (2022) and Nyangena et al. (2020) reported that freeze-drying and oven-drying led to further reduction of TVC as observed in this study. However, it should be noted that the application of just a drying treatment (without blanching) is not as effective as the application of blanching (Larouche et al., 2019; Nyangena et al., 2020).

4. Conclusion

This study provides further insights into how different processing methods can influence the quality and safety of *T. molitor* larvae. It is essential that applied processing methods can guarantee that insects exhibit microbial safety as well as good nutritional and sensory characteristics for further incorporation into food products. It is also important that methods can be applied at an industrial scale without prohibitive energy and monetary costs.

In this regard, this study further demonstrated the importance of applying blanching treatments to insects to not only assure microbial safety but also improve color characteristics and macro-nutrient composition. Steam-blanching can function as an alternative to immersion-blanching, particularly to assure microbial safety. Application of drying methods had a negative effect on the color of the larvae, but it further improved microbial safety. Drying with a freeze dryer

provided the best color properties, but it led to the highest TVC and Yeasts & Molds content. Drying with an oven or microwave can function as good alternatives to the freeze dryer while also requiring less time, energy and money. Furthermore, the color of oven-dried or microwave-dried *T. molitor* samples can be further improved by applying conditions with a lower duration. Additionally, results from our study also support that maintaining insects frozen until they are dried is the best approach to ensure better color properties. The results of this study can be applied at an industrial level, since they show that edible insects' processing can be improved, in terms of productivity as well as energy and water consumption, through the application of alternative blanching (steam) and drying (oven and microwave) methods, without negatively impacting the macro-composition and microbial load of the insects.

Nevertheless, this study also presents some limitations related to the level of characterization that was performed. For instance, besides assessing macro-composition it would be helpful to also assess amino-acid/fatty acid profile and micronutrient (e.g., minerals and vitamins) composition. Furthermore, it would also be interesting to assess the stability of the microbial load for a longer period, above 3 months, and to assess the presence of pathogenic bacteria. Future studies should also focus on the effects of processing conditions on the sensory attributes of edible insects, which is a poorly understood topic. Additionally, the effect of different steam-blanching conditions (steam temperature, time or pressure application) on the nutritional composition, techno-functional properties and microbial load of edible insects should be assessed in future studies, considering that these blanching methods have been rarely applied with edible insects.

CRediT authorship contribution statement

José Carlos Ribeiro: Conceptualization, Formal analysis, Investigation, Writing – original draft. **João Pedro Marques:** Investigation, Writing – original draft. **Tânia R. Fernandes:** Investigation, Writing – review & editing. **Manuela Estevez Pintado:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Susana M.P. Carvalho:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Luís Miguel Cunha:** Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

Authors José Carlos Ribeiro, João P. Marques, Tânia R. Fernandes, Manuela Estevez Pintado, Susana M.P. Carvalho and Luís Miguel Cunha, of the manuscript *Effect of blanching, storage and drying conditions on the macro-composition, color and safety of mealworm Tenebrio molitor larvae,* submitted to LWT – Food Science & Technology, declare there is no conflict of interests related with the work here reported.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115646.

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