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Short communication

Effect of blanching followed by refrigerated storage or industrial microwave drying on the microbial load of yellow mealworm larvae (*Tenebrio molitor*)

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ABSTRACT

Yellow mealworm larvae (*Tenebrio molitor*) are being introduced into Western food products. The effect of blanching, followed by either chilled storage or industrial microwave drying, on microbial counts of the larvae was investigated. Whatever time applied (10, 20 or 40 s), considerable log reductions were obtained after blanching (total viable count, Enterobacteriaceae, lactic acid bacteria, yeasts and molds and psychrotrophs), except for aerobic endospores. No major growth was observed during subsequent chilled storage for 6 days. Total viable counts were below 3.5 ± 0.3 log cfu/g for all samples. When blanching for 40 s was followed by industrial microwave drying, drying for 8, 10 or 13 min did not yield larvae with a water activity below 0.60, which is necessary to eliminate all microbial growth. Drying times of 16 or 20 min yielded average water activities of 0.16 and 0.23, respectively. The number of vegetative cells was reduced to a large extent by blanching plus drying, but the number of bacterial endospores only slightly. Total viable counts were maximally 3.4 ± 0.8 log cfu/g for all samples. Bacterial endospores were the most resistant to the processing technologies investigated.

1. Introduction

As edible insects are being introduced into the Western food pattern, several ways to process insects into food products are currently being explored. After rearing, mealworms are typically frozen to gently kill them and additionally they may be freeze-dried. Those practices enable long term storage and transport, thus facilitating the supply chain management of the insects. However, they are both expensive technologies, freezing especially with respect to the concomitant frozen transport and freeze-drying as to investment as well as operational costs (energy consumption). Both unit operations add to the cost of edible insects as a raw material for food companies. Alternative ways for processing need to be explored, which are preferably also more sustainable. Alternatives are only valuable when they are feasible on an industrial scale, when they are payable and, last but not least, when they result in intermediate or end products of good nutritional and microbial quality. The aim of this work was to consider chilling and microwave drying of mealworms as alternatives for freezing and freeze-drying. The focus was on the effect of both technologies on the microbial load of mealworm larvae. The impact of chilling and microwave drying on the microbiota of mealworms has not been studied before. The microbial load of fresh mealworms after rearing is high, with total viable counts generally being about 7–8 log cfu/g (Klunder, Wolkers-Rooijackers, Korpela, & Nout, 2012; Rumpold et al., 2014; Stoops et al., 2016). Therefore, chilling and microwave drying were preceded in this study by blanching. In Belgium, the Federal Agency for the Safety of the Food Chain postulates that a heating step, such as blanching, is necessary to reduce microbial numbers on insects before they are placed on the market (Ngonlong Ekendé, Bergen, & Keppens, 2016). However, no information is available on log reductions that can be obtained for specific time and temperature combinations.

In this study, fresh mealworm larvae were first blanched and then either stored in refrigerated conditions or microwave dried. In

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the experiments involving blanching and chilled storage, several blanching times were included. In the experiments on blanching followed by microwave drying, one single blanching time was considered but several drying times were applied.

2. Materials and methods

2.1. Insect samples

Living insects were purchased and transported to the laboratory and stored until analysis at ambient temperature. The larvae used for blanching and chilled storage were obtained from an eco-shop in Antwerp (Belgium, three batches on one occasion). Those used for blanching and microwave drying were purchased from an insect raiser in Tessenderlo (Belgium, two batches on two occasions).

2.2. Blanching and chilled storage

Duplicate aliquots of 400 g larvae were blanched by transferring them into 4 l of boiling water and keeping them in the boiling water for respectively 10, 20 or 40 s. After blanching, they were transferred immediately into 9 l sterile water using an autoclaved sieve. The water was chilled in advance in an ice water bath. The larvae were kept there for 60 s and drained by placing them in a sterile sieve for 30 s. Prior to refrigerated storage, for each blanching time two plastic bags with each 100 g larvae were prepared (VAC090 PA/PE 20/70, thickness 80 μm, width 20 cm, Euralpack, Belgium). The bags were sealed (including air) using a packaging machine (C 200, Multivac, Belgium). They were kept for 6 days in a home type refrigerator (Miele, Belgium) with set point 3 °C. The temperature during storage was monitored by a logger (Escort Data Logger, iLog). Microbial counts were performed on larvae before blanching, after blanching and after refrigerated storage. The untreated insects were anesthetized before analysis by incubating them in 100% nitrogen gas (Praxair, Belgium) for at least 1.5 min. Prior to microbiology analyses, samples of 30 g were taken aseptically from each batch and ground using an ethanol sterilized hand held mixer (Bosch CNHR 25, speed 12, 2 min) as described previously (Stoops et al., 2016). Hence, microorganisms on the surface as well as in the intestine were counted, as larvae are consumed or processed entirely.

2.3. Blanching and industrial microwave drying

For each drying time, an aliquot of 1000 g larvae was blanched by transferring them into 4 l of boiling water and keeping them in the boiling water for 40 s. Then they were chilled and drained as mentioned above. They were transported (45 min) to the microwave drying facility at ambient temperature. Microwave drying was performed in a MEAMDRY S 32 belt dryer (with 16 microwave sources of 2 kW and a conveyer length of 4 m, MEAM, Herk-de-Stad, Belgium). The larvae were placed on the conveyer in a layer of 1.5 cm. Larvae were subjected to different drying times and aliquots of 500 g were pooled per drying time. The rate of the conveyer and the number of passages of the sample through the dryer were selected to reach a drying time of 8, 10, 13, 16 or 20 min. The temperature in the dryer was registered by a sensor (Raytek, MI 3 type LTS, USA). After drying, the larvae were transferred into sterile bags and immediately transported to the laboratory and investigated. Samples of 30 g were taken aseptically from each batch, ground as described above and used for determination of moisture content, water activity and microbial counts.

2.4. Moisture content and water activity analysis

The moisture content was calculated from the weight loss of a sample of 2–3 g larvae during oven drying overnight at 105 °C. Water activity measured on a 7 g aliquot of each sample (LabMaster aw, Novasina, Lachen, Switzerland) until a stable water activity and temperature value (20 °C) persisted for 15 and 5 min, respectively.

2.5. Microbiological analysis

Of each ground subsample, 5 g was brought into a stomacher bag together with 45 g of peptone physiological salt solution (PPS, 0.85% NaCl, 0.1% peptone, Biokar Diagnostics, Beauvais, France). After homogenization for 60 s in a Bagmixer® (Interscience, Saint Nom, France), a tenfold dilution series was prepared and plated on different agar media (Biokar diagnostics) using the pour-plate technique, according to the ISO standards assembled by Dijk et al. (2015). Total viable mesophilic counts were determined on Plate Count Agar (PCA) after incubation for 72 h at 30 °C, lactic acid bacteria (LAB) on de Man, Rogosa & Sharpe agar for 72 h at 30 °C, Enterobacteriaceae on Violet Red Bile Glucose agar for 24 h at 37 °C, yeasts and molds on spread plates of Dichloran Rose-Bengal Chloramphenicol Agar after incubation for 5 days at 25 °C. Aerobic bacterial endospores were determined after a pasteurization treatment of the 10^{-1} dilution at 80 °C for 10 min, followed by dilution and incubation on PCA for 48 h at 37 °C. Psychrotrophs were counted on PCA for 10 days at 6.5 °C.

2.6. Statistical analysis

SPSS Statistics 23 (IBM, New York, USA) was used. Differences between initial microbial and intrinsic values and values obtained after treatment within the same batch were investigated with One-way ANOVA, followed by the Duncan Post-Hoc test. Initial values from different batches from the same raiser were compared as well, with an identical One-way ANOVA test for the chilling experiment and an Independent-samples T-test for the drying experiment. All tests were performed with a significance level of 0.05.

3. Results and discussion

3.1. Blanching and chilled storage

For vegetables and fruits, blanching is mainly applied as a pretreatment to inactivate enzymes and to reduce the microbial load prior to further storage or processing (Fellows, 2009; Xu et al., 2012). In our study, the main purpose of blanching mealworms was to reduce their microbial load prior to further storage or processing. As presented in Table 1, the microbial counts before blanching were very similar for the three batches investigated. No statistical differences for the individual counts were observed between batches, except for the LAB (although the range was only 0.5 log cfu/g) and the psychrotrophs (ranging between 6.0 and 7.2 log cfu/g). The total count, the number of Enterobacteriaceae and the number of LAB reported by Stoops et al. 2016, who used similar procedures for sample treatment and counts, were in line with our counts. However, Stoops et al. (2016) found a highly variable number of aerobic spores (3.5, <1.0 and <1.0 in three batches), whereas our data ranged between 2.6 and 3.1 log cfu/g. Quite high numbers of psychrotrophs (ranging between 6.0 and 7.2 log cfu/g) were observed in our study, demonstrating the potential for microbial spoilage when storing fresh mealworms in refrigerated conditions.

Blanching resulted in a very pronounced and also statistically significant reduction of all types of counts and for all blanching