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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 sec), 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 six days. Total viable counts were below 3.5 ± 0.3 log cfu/g for all samples. When blanching for 40 sec 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.

Keywords

Mealworm larvae; blanching; chilling; microwave drying; microbial counts

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 to 8 log cfu/g (Klunder et al., 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 et al., 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 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 sec. 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 sec and drained by placing them in a sterile sieve for 30 sec. 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 μ m, width 20 cm, Euralpack, Belgium). The bags were sealed (including air) using a packaging machine (C 200, Multivac, Belgium). They were kept for six 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 sec. 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 and 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 to 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 five 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 times, except for the spores. For total counts, log reductions of 4.4, 6.4 and 5.6 log cycles were observed for blanching times of 10, 20 and 40 sec respectively. The data do not show a relation between blanching time and magnitude of reduction. Even blanching during 10 sec decreased microbial counts below the detection limit, except for the total count and the spores. The effect of blanching on the spore count was not consistent: blanching during 10 sec yielded similar spore counts after the treatment than before, whereas blanching during 40 sec resulted in a numerical reduction with 0.8 log cfu/g,

and blanching at 20 sec caused a substantial and unexplainable rise in the spore count. Similarly, Klunder et al. (2012) reported 2.1 and < 1 log cfu/g spores on fresh mealworms and larvae boiled for 10 min, respectively. In our study the microbial effect of shorter blanching times was studied. If effective in reducing the microbiota, shorter times may be more suitable for industrial processing of mealworms and more nutrients such as vitamins might survive. Hence, although in our study the number of spores before blanching was lowest when all microbial subgroups are compared, after blanching the spore count was highest. That suggests that blanching causes the microbial community of mealworm larvae to be reduced to more or less bacterial spores only.

After blanching, the mealworm larvae were packaged in air and stored for six days in refrigerated conditions. The temperature during storage was $3.7 \pm 1.7^{\circ}\text{C}$. The data in Table 1 show that there was no microbial growth during cooled storage, except for a slight growth of mesophilic and psychrotropic organisms in batch III (i.e. 40 sec blanching). That is surprising, as one would expect that the longest blanching time reduces the microbiota the most and results in the lowest growth during chilled storage. For all three batches, the spore count was reduced during chilled storage and the reductions were statistically significant for batches II and III. Possibly part of the spores germinated during chilled storage and contributed to other counts. It can be stated that after blanching, mealworms can be kept for (at least) six days without spoilage under refrigerated conditions. Even though some numbers can increase slightly, they stay far below the level of 7 log cfu/g which is considered as a general spoilage level for foods (Sperber and Doyle, 2009).

3.2. Blanching and industrial microwave drying

Fresh mealworm larvae were characterized by water activity values of 0.96 and 0.95 for batch I and II respectively (Table 2). The results did not differ statistically between the two batches

($P = 0.220$). In contrast, the moisture contents differed between the two batches ($P=0.000$). Whereas optimization of industrial microwave drying was not the main objective, several drying times were tested, as the drying period necessary to obtain a microbiologically stable end product was not known for the installation used. Water activity measurements (Table 2) pointed out that 8 or 10 min drying were not enough to reduce the initial values to values below 0.60 (see data obtained for batch II). Values below 0.60 are aimed at, since microbial growth is not possible below that limit (Jay et al., 2005). A drying time of 13 min was not sufficient to obtain a microbiologically stable end product as it yielded a water activity slightly above 0.60 (0.67 ± 0.10). When drying for 16 or 20 min, extremely low water activities and moisture contents were recorded. The larvae had a dark color after the treatment and were considered to be overprocessed. Increasing drying times lead to lower water activities. Between 13 and 16 min a sudden drop seems to have occurred in the water activity and moisture content. Several preliminary tests on microwave drying at pilot scale also showed a sudden drop in both parameters in function of drying time (data not shown). In batch II, unblanched as well as blanched samples were subjected to a drying time of 10 min. Blanching as a pretreatment did not affect the moisture content and water activity obtained. The total counts and spore, Enterobacteriaceae and LAB counts for fresh mealworms found in the drying experiment (Table 3) were very comparable to those for fresh larvae recorded in the chilling experiment (Table 1), despite the fact that the larvae were purchased from a different supplier. The effect of 40 sec blanching (as such, without subsequent treatment) can also be compared for the two experiments. The total counts in Table 3 reveal a slightly higher reduction by 40 sec blanching (6.3 in batch I and 5.8 in batch II) than the total counts in Table 1 (5.6 in batch III). That can possibly be explained by that fact that on fresh larvae the number of spores, which are most heat resistant, was slightly higher in batch III (3.1 log cfu/g, see Table 1) than in batches I and II (2.3 and 2.6 log cfu/g, see Table 3).

As to the effect of microwave drying on microbial numbers (Table 3), we first consider the samples that were subjected to a blanching step before drying. A longer drying times resulted in a stronger reductions of the total counts, reaching a plateau: log reductions of 4.7, 4.8, 6.0, 6.5 and 6.5 were achieved when applying 8, 10, 13, 16 and 20 min drying, respectively. This was related to the temperature of the larvae measured at the exit of the dryer, which was 55, 47, 71 and 72°C when drying for 10, 13, 16 and 20 min, respectively. Likewise, Yaghmaee and Durance (2007) and Jeevitha et al. (2016) reported a gradual killing of the microbial population in other food matrices in function of exposure time to microwaves. For Enterobacteriaceae, LAB and yeasts and molds, counts after blanching followed by drying were below or very close to the detection limit (1.0 and 2.0 log cfu/g for bacterial and fungal counts, respectively). Survival was lowest for the Enterobacteriaceae, which can be explained by the fact that in general, they are quite sensitive to heat treatments compared to other microbial subgroups in foods (Baylis et al., 2001). For bacterial endospores, blanching and drying only yielded very low log reductions, ranging between 0.3 and 1.0. Whereas on fresh larvae, the numbers of spores were considerably lower than those of Enterobacteriaceae, LAB and fungi, on blanched and dried larvae, their counts were in the same range (i.e. between 1.3 and 2.3) as the counts of the other subgroups (i.e. between < 1.0 and 2.8).

When microwave drying was applied as a sole treatment in batch II, for all counts statistically significant reductions were recorded ($P=0.000$, 0.009, 0.000, 0.000, 0.000 for total, spore, Enterobacteriaceae, LAB and fungal counts respectively). The microbial inactivation of microwaves was due to a temperature increase in the matrix, likely combined with electromagnetical effects (Banik et al., 2003; Celandroni et al. 2004; Kang and Kato, 2014).

4. Conclusions

The objective of this study was to investigate chilling and microwave drying of blanched mealworm larvae from a microbial perspective. Regardless of treatment times, blanching alone or blanching plus microwave drying represent a pasteurization treatment, i.e. killing vegetative cells but not or hardly spores. Blanched mealworms can be stored in refrigerated conditions for (at least) six days without substantial microbial growth. Whatever way edible insects are processed and whatever insect species is considered, bacterial spores and their survival need special attention.

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Table 1

Microbial counts of mealworm larvae before and after blanching for different treatment times and after subsequent chilled storage of six days at $3.7 \pm 1.7^\circ\text{C}$. Data are the mean values of three to six replicates $\pm$ standard deviation.

Batch	Treatment	Microbial counts (log cfu/g)					
		Total aerobic count	Aerobic bacterial endospores	Enterobacteriaceae	Lactic acid bacteria	Yeasts and molds	Psychrotrophic aerobic count
I	None (Initial count)	$7.9 \pm 0.3^{a, A}$	$2.6 \pm 0.3^{a, A}$	$7.3 \pm 0.5^{a, A}$	$7.4 \pm 0.2^{a, A}$	$3.8 \pm 0.5^{a, A}$	$7.2 \pm 0.4^{a, A}$
	10 sec blanching	3.5 ± 0.8^b	2.8 ± 1.0^a	$< 1.0 \pm 0.0^b$	$< 1.0 \pm 0.0^b$	$< 2.0 \pm 0.0^b$	$< 1.0 \pm 0.0^b$
	Chilled storage	2.7 ± 0.2^c	2.3 ± 0.1^a	$< 1.0 \pm 0.0^b$	1.3 ± 0.4^b	$< 2.0 \pm 0.0^b$	1.1 ± 0.1^b
II	None (Initial count)	$8.2 \pm 0.7^{a, A}$	$2.8 \pm 0.4^{a, A}$	$7.5 \pm 0.6^{a, A}$	$7.0 \pm 0.1^{a, B}$	$3.5 \pm 0.2^{a, A}$	$6.0 \pm 0.2^{a, B}$
	20 sec blanching	1.8 ± 0.4^b	4.7 ± 0.7^b	$< 1.0 \pm 0.0^b$	$< 1.0 \pm 0.0^b$	$< 2.0 \pm 0.0^b$	$< 1.0 \pm 0.0^b$
	Chilled storage	2.9 ± 0.2^b	2.4 ± 0.1^a	1.2 ± 0.3^b	$< 1.0 \pm 0.0^b$	$< 2.0 \pm 0.0^b$	1.3 ± 0.1^b
III	None (Initial count)	$7.6 \pm 0.4^{a, A}$	$3.1 \pm 0.1^{a, A}$	$7.1 \pm 0.9^{a, A}$	$6.9 \pm 0.2^{a, B}$	$3.5 \pm 0.4^{a, A}$	$6.5 \pm 0.6^{a, A, B}$
	40 sec blanching	2.0 ± 0.4^b	2.3 ± 0.1^a	$< 1.0 \pm 0.0^b$	1.5 ± 0.8^b	$< 2.0 \pm 0.0^b$	$< 1.0 \pm 0.0^b$
	Chilled storage	3.5 ± 0.3^c	1.4 ± 1.1^b	1.1 ± 0.1^b	1.3 ± 0.3^b	$< 2.0 \pm 0.0^b$	2.8 ± 0.8^c

^{a,b,c}Mean values per treatment with the same superscript within the same batch are not statistically different ($p > 0.05$).

^{A,B}Mean initial counts from different batches with the same superscript are not statistically different ($p > 0.05$).

Table 2

Moisture content and water activity of mealworm larvae without treatment, after 40 sec blanching, 10 min microwave drying, and after 40 sec blanching followed by microwave drying for 16 and 20 min (batch I) and for 8, 10 and 13 min (batch II). Data are the mean values of three to six replicates $\pm$ standard deviation.

Batch	Treatment	Intrinsic properties	
		Moisture content (%)	Water activity (-)
I	None (Initial value)	62.81 $\pm$ 0.34 ^{a,A}	0.96 $\pm$ 0.00 ^{a,A}
	Only 40 sec blanching	65.61 $\pm$ 0.57 ^b	0.96 $\pm$ 0.10 ^a
	40 sec blanching + 16 min drying	0.66 $\pm$ 0.37 ^c	0.16 $\pm$ 0.07 ^b
	40 sec blanching + 20 min drying	1.06 $\pm$ 0.44 ^c	0.23 $\pm$ 0.06 ^c
II	None (Initial value)	66.57 $\pm$ 0.19 ^{a,B}	0.95 $\pm$ 0.01 ^{a,A}
	Not blanched, only 10 min drying	22.75 $\pm$ 3.49 ^b	0.85 $\pm$ 0.02 ^b
	Only 40 sec blanching	70.44 $\pm$ 0.14 ^a	0.96 $\pm$ 0.00 ^a
	40 sec blanching + 8 min drying	31.33 $\pm$ 2.88 ^b	0.90 $\pm$ 0.01 ^{a,b}
	40 sec blanching + 10 min drying	26.18 $\pm$ 10.94 ^b	0.85 $\pm$ 0.06 ^b
	40 sec blanching + 13 min drying	10.52 $\pm$ 4.06 ^c	0.67 $\pm$ 0.10 ^c

^{a,b,c}Mean values per treatment with the same superscript within the same batch are not statistically different ($p > 0.05$).

^{A,B}Mean initial counts from different batches with the same superscript are not statistically different ($p > 0.05$).

314 **Table 3**

315 Microbial counts of mealworm larvae without treatment, after 40 sec blanching, after 10 min microwave drying, and after 40 sec blanching
 316 followed by microwave drying for 16 and 20 min (batch I) and for 8, 10 and 13 min (batch II). Data are the mean values of three to six replicates
 317 $\pm$ standard deviation.

Batch	Treatment	Microbial counts (log cfu/g)				
		Total aerobic count	Aerobic bacterial endospores	Enterobacteriaceae	Lactic acid bacteria	Yeast and molds
I	None (Initial count)	$7.8 \pm 0.3^{a,A}$	$2.3 \pm 0.2^{a,A}$	$7.4 \pm 0.4^{a,A}$	$6.8 \pm 0.2^{a,A}$	$5.0 \pm 0.4^{a,A}$
	Only 40 sec blanching, not dried	1.5 ± 0.4^b	1.4 ± 0.2^b	$< 1.0 \pm 0.0^b$	1.3 ± 0.6^b	2.8 ± 0.5^b
	40 sec blanching + 16 min drying	1.3 ± 0.2^b	1.3 ± 0.2^b	$< 1.0 \pm 0.0^b$	$< 1.0 \pm 0.0^b$	$< 2.0 \pm 0.0^c$
	40 sec blanching + 20 min drying	1.3 ± 0.2^b	1.4 ± 0.2^b	$< 1.0 \pm 0.0^b$	$< 1.0 \pm 0.0^b$	2.1 ± 0.1^c
II	None (Initial count)	$8.1 \pm 0.0^{a,A}$	$2.6 \pm 0.5^{a,A}$	$7.4 \pm 0.3^{a,A}$	$7.2 \pm 0.0^{a,B}$	$4.8 \pm 0.3^{a,A}$
	Not blanched, only 10 min drying	$2.9 \pm 0.7^{b,c}$	$1.9 \pm 0.2^{b,c}$	2.1 ± 0.1^b	2.3 ± 0.5^b	$< 2.0 \pm 0.0^b$
	Only 40 sec blanching, not dried	$2.3 \pm 0.1^{b,c}$	$2.3 \pm 0.1^{a,b}$	$< 1.0 \pm 0.0^c$	1.3 ± 0.1^b	2.2 ± 0.3^b
	40 sec blanching + 8 min drying	3.4 ± 0.8^b	$1.9 \pm 0.3^{b,c}$	$< 1.0 \pm 0.0^c$	1.8 ± 0.8^b	2.2 ± 0.4^b
	40 sec blanching + 10 min drying	$3.3 \pm 1.3^{b,c}$	$1.9 \pm 0.3^{b,c}$	$< 1.0 \pm 0.0^c$	2.0 ± 1.5^b	$< 2.0 \pm 0.0^b$
	40 sec blanching + 13 min drying	2.1 ± 0.4^c	1.7 ± 0.1^c	$< 1.0 \pm 0.0^c$	$< 1.0 \pm 0.0^b$	$< 2.0 \pm 0.0^b$

318 ^{a,b,c}Mean values per treatment with the same superscript within the same batch are not statistically different ($p > 0.05$).

319 ^{A,B}Mean initial counts from different batches with the same superscript are not statistically different ($p > 0.05$).