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Microbial counts of mealworm larvae (*Tenebrio molitor*) and crickets (*Acheta domesticus* and *Gryllodes sigillatus*) from different rearing companies and different production batches

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ABSTRACT

The rising interest in insects for human consumption and the changing regulations in Europe require a profound insight into the food safety of insects reared and sold in Western society. The microbial quality of edible insects has only been studied occasionally. This study aimed at generating an overview of intrinsic parameters (pH, water activity and moisture content) and microbial quality of fresh mealworm larvae and crickets for several rearing companies and for several batches per rearer. In total, 21 batches obtained from 7 rearing companies were subjected to analysis of intrinsic parameters, a range of plate counts and presence-absence tests for *Salmonella* spp. and *Listeria monocytogenes*. The microbial counts of the fresh insects were generally high. Different rearing batches from a single rearing company showed differences in microbial counts which could not be explained by variations in intrinsic properties. The largest variations were found in numbers of bacterial endospores, psychrotrophs and fungi. *Salmonella* spp. and *L. monocytogenes* were not detected in any of the samples. Altogether, our study shows that large variations were found between batches from individual rearers. As a consequence, no overall differences between rearers could be observed.

1. Introduction

Interest in human consumption of edible insects (entomophagy) in Western countries is increasing (Caparros Megido et al., 2014; Mlcek et al., 2014) and more and more insect-based food products are being marketed. Compared with Asian, African, Oceanian and Latin American regions, Western society has no history of insect consumption (Siemianowska et al., 2013; van Huis, 2013; van Huis et al., 2013; Yen, 2015). However, insects are a promising and valuable alternative to conventional protein sources such as meat. They provide an opportunity to meet the increased protein demand of the growing world population (Mlcek et al., 2014; Premalatha et al., 2011; van Huis et al., 2013). Moreover, rearing insects for food has a smaller ecological footprint compared to traditional animal husbandry (Oonincx and de Boer, 2012; Oonincx et al., 2010; van Huis et al., 2013).

Insects will be considered as Novel Food in Europe starting from January 2018, as stated in the new Regulation (EU) 2015/2283 on novel foods. Hence, more research data on the microbial quality of edible insects reared in Europe are necessary to support risk assessments performed by the European Food Safety Authority (EFSA) (Belluco et al., 2013). Additionally, more quantitative data concerning the microbial quality will be needed in order to establish microbial criteria for edible insects in the future, similar to existing criteria for other food products (Regulation (EC) N° 2073/2005 on microbiological criteria for foodstuffs).

Presently, the nutrient composition of several insect species has already been studied extensively (Finke, 2002; Nowak et al., 2016; Rumpold and Schlüter, 2013a; Sánchez-Muros et al., 2014; Siemianowska et al., 2013). Microbiological data, however, are only scarcely available, as highlighted in a recent EFSA opinion (EFSA Scientific Committee, 2015). Moreover, the few studies available containing microbiological data (Giaccone, 2005; Grabowski et al., 2014; Klunder et al., 2012; Rumpold et al., 2014) do not include analyses of different production batches or insects from different rearing companies. So far, there is only one study including different production batches (Stoops et al., 2016), but they originate from only one rearing company. None of the studies available contain data on intrinsic properties of edible insects, such as pH and water activity (a_w), although those

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factors have an important impact on the growth and survival of microorganisms (Madigan et al., 2009) and need to be taken into account when considering insects as a food matrix.

The objective of this study is to investigate the microbial load and intrinsic properties of fresh mealworm larvae (*Tenebrio molitor*) and crickets (*Acheta domesticus* and *Gryllodes sigillatus*) as a food product. In order to obtain a generalized view, different production batches and rearing companies were included in the study. pH, moisture content and a_W were determined, as well as a range of plate counts and presence-absence tests for pathogens typically determined for foods.

2. Material and methods

2.1. Study materials

Three insect species commonly reared for human consumption were investigated: *Acheta domesticus* (house cricket), *Gryllodes sigillatus* (banded cricket) and larvae of *Tenebrio molitor* (mealworm). Samples were obtained from seven rearing companies in Belgium and the Netherlands, including five companies specialized in rearing for human consumption and two companies for pet food. For each company, three production batches (i.e. rearing cycles) were sampled between March and December 2015, resulting in 21 batches studied, consisting of 12 mealworm and 9 cricket batches (Table 1).

2.2. Sampling and sample preparation

Samples of fully grown and living insects ready for consumption were transported to the laboratory at ambient temperature and immediately processed upon arrival. Prior to analysis, insects were sedated by cooling (±4 °C, 1 h). Subsequently, three subsamples of 30 g were taken aseptically from each batch and pulverized (Bosch CNHR 25, max speed) as described previously (Stoops et al., 2016).

2.3. Intrinsic properties

All subsamples were subjected to measurements of pH, water activity (a_w) and moisture content. pH was measured in threefold using a digital pH meter (Portamess 911, Knick, Berlin, Germany with SI analytics electrode, Mainz, Germany). A single a_w measurement was performed on a 7 g aliquot of each subsample using a water activity meter (LabMaster a_w, Novasina, Lachen, Switzerland), until water activity and temperature (20 °C) were stable for 15 and 5 min, respectively. Moisture content was calculated from the weight loss of 2 to 3 g from each subsample after oven-drying overnight at 105 °C.

2.4. Microbial plate counts

Since it was not clear whether pulverization of the insects before homogenization would affect microbial counts, a preliminary experiment was executed. Several counts (mesophilic aerobic count, aerobic endospores and Enterobacteriaceae, see below) were determined using a procedure with and without pulverization (as described in Section 2.2). Both approaches were performed on five subsamples of a mealworm sample obtained from company 4 (Table 1). Because pulverization was found to be necessary for optimal extraction of microorganisms from their matrix (see Section 3.1), the step was included in all further analyses.

To obtain a primary dilution, 5 g of each pulverized subsample and 45 g of peptone physiological salt solution (PPS, 0.85% NaCl, 0.1% peptone, Biokar Diagnostics, Beauvais, France) were mixed together in a stomacher bag. After homogenization for 60 s in a Bagmixer® (Interscience, Saint Nom, France), a tenfold dilution series was prepared and plated using the pour-plate technique, according to the ISO standards assembled by Dijk et al. (2015). Bacterial endospores and yeasts and moulds were determined according to Dijk et al. (2007). Total viable mesophilic and psychrotrophic aerobic counts were assessed after aerobic incubation on Plate Count Agar (PCA, Biokar diagnostics) for respectively 72 h at 30 °C and 10 days at 6.5 °C. Lactic acid bacteria (LAB) were incubated on de Man, Rogosa & Sharpe agar (MRS, Biokar diagnostics) for 72 h at 30 °C, Enterobacteriaceae on Violet Red Bile Glucose agar (VRBG, Biokar diagnostics) for 24 h at 37 °C, and yeasts and moulds on Oxytetracycline Glucose Agar (OGA, Biokar diagnostics) supplemented with oxytetracycline (50 mg/550 ml OGA, Biokar diagnostics) for 5 days at 25 °C. Aerobic bacterial endospores were determined on PCA for 24 h at 37 °C after a pasteurisation treatment of the 10^{-1} dilution at 80 °C for 10 min.

2.5. Pathogen detection

Pulverized samples were also used for detection of *Salmonella* spp. and *Listeria monocytogenes*. Detection of *Salmonella* spp. was performed

Table 1
Sample information.

Sample ID	Rearing company	Batch	Sampling month (2015)	Insect type	Species	Purpose (human/pet food)
MW 1.1	1	1	March	Mealworm	*T. molitor*[a]	Human food
MW 1.2	1	2	May	Mealworm	*T. molitor*	Human food
MW 1.3	1	3	September	Mealworm	*T. molitor*	Human food
MW 2.1	2	1	March	Mealworm	*T. molitor*	Human food
MW 2.2	2	2	June	Mealworm	*T. molitor*	Human food
MW 2.3	2	3	October	Mealworm	*T. molitor*	Human food
MW 3.1	3	1	May	Mealworm	*T. molitor*	Pet food
MW 3.2	3	2	July	Mealworm	*T. molitor*	Pet food
MW 3.3	3	3	November	Mealworm	*T. molitor*	Pet food
MW 4.1	4	1	July	Mealworm	*T. molitor*	Pet food
MW 4.2	4	2	August	Mealworm	*T. molitor*	Pet food
MW 4.3	4	3	September	Mealworm	*T. molitor*	Pet food
CR 1.1	5	1	March	Cricket	*A. domesticus*[b]	Human food
CR 1.2	5	2	June	Cricket	*A. domesticus*	Human food
CR 1.3	5	3	September	Cricket	*A. domesticus*	Human food
CR 2.1	6	1	April	Cricket	*A. domesticus*	Human food
CR 2.2	6	2	July	Cricket	*A. domesticus*	Human food
CR 2.3	6	3	October	Cricket	*A. domesticus*	Human food
CR 3.1	7	1	August	Cricket	*G. sigillatus*[c]	Human food
CR 3.2	7	2	October	Cricket	*G. sigillatus*	Human food
CR 3.3	7	3	December	Cricket	*G. sigillatus*	Human food

[a] *T.: Tenebrio.*
[b] *A.: Acheta.*
[c] *G.: Gryllodes.*