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EFFECT OF DIFFERENT STORAGE METHODS ON THE MICROBIOLOGICAL QUALITY OF THE INSECT DRY POWDER MADE FROM MEALWORM (*TENEBRIO MOLITOR*, L.)

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Abstract

Entomophagy as an alternative way of eating in the Czech Republic is gaining more and more supporters. For this reason, we have focused on the microbial quality of the insect dry powder made from *Tenebrio molitor* during storage. In the samples were determined the major groups of microorganisms by standard procedures. It is the total number of microorganisms, aerobic microorganisms thermoresistant, *E. coli* and other coliform bacteria, micromycetes and bacteria of the genus *Enterococcus*. The highest increase of total microorganisms occurred after five months storage in the sample stored in a paper bag and cold, where their number reached a value of 2.870 log CFU/g. For the same sample, coliform bacteria at the rate of 1.839 log CFU/g were also detected. The other monitored groups of microorganisms were in the samples at most in the order of tens of CFU/g.

Keywords: mealworms, entomophagy, the total number of microorganisms, edible insects, *Tenebrio molitor*

INTRODUCTION

Insects as a new type of food gain more and more attention in the Western countries. It is an alternative source of high-quality protein. Species commonly used for human nutrition are referred to as edible insects. This term can be used to describe all insect species whose consumption does not adversely affect the health of the consumer (Borkovcová, 2009).

Interest in edible insects is also on the part of the European Union. According to EC Regulation 2017/893 (from July 1st, 2017), selected species of insects kept for the production of processed animal proteins are considered to be livestock and can be used as a safe material for production of feed for pets, fur animals and for feeding fish. Another EC Regulation 2015/2283 (from 25 November 2015) about novel food classifies selected insect species as novel foods. This is a significant legislative shift that moves us closer to the legal use of insects for food production. This is precisely why we have focused on the microbial quality of the insect dry powder made from mealworm (*Tenebrio molitor*).

Generally, insect-associated microorganisms can be divided into two basic groups. Microorganisms naturally occurring on the surface of the body and in the digestive system of insects, and microorganisms that contaminate insects during breeding and processing. Microorganisms present in the digestive system of insects are essential for its metabolism. Since food insects are also processed with intestinal content, it is important to focus on knowledge of edible microbial insects, especially because the pathogenic micro-organisms that may endanger the health of the consumer may be present in the above-mentioned groups of insect-associated microorganisms (EFSA, 2015).

The aim of this work was to determine the amount of microorganisms in the product from dried and crushed worms *Tenebrio molitor* (the insect dry powder) and to determine the most suitable conditions for its storage.

MATERIALS AND METHODS

For the purpose of this experiment, the larvae of the species *Tenebrio molitor* were purchased for production of the insect dry powder. The larvae were purchased from local farm. The Age of the larvae were between the last instar and pupa. The farm did not disclose the compound feed composition. The larvae were kept in an empty plastic container for 48 to 72 hours for perfect clean out. Prior to processing, the larvae were killed by immersion in boiling water for 1 minute. After killing, the larvae were dried in a hot-air oven at 120 °C for 1 hour. These parameters were selected after testing various combinations of temperatures and times, because they achieved the best quality of dried worms. The dried larvae were milled to powder.

A 5 g sample was collected from the freshly milled insect dry powder for microbiological analysis. The remaining material was divided into three different types of packaging. Specifically, paper bags, plastic samplers and samples vacuum-wrapped in foil. Each package type was stored in a refrigerator at 4–6 °C with 90% humidity and at room temperature 22–23 °C.

Samples for microbiological analysis were collected at intervals of 2 to 3 months. The collected samples were inserted into sterile centrifuge tubes, supplemented with 45 ml of sterile saline solution and shaken for 1 minute. The following groups of microorganisms were determined by standard procedures: the total counts of microorganisms (TCM) on PCA (Biokar Diagnostics, France) at 30 °C for 72 hours, aerobic thermoresistant microorganisms (after preheating at 85 °C for 10 minutes on PCA Biokar Diagnostics, France) at 30 °C for 72 hours, *E. coli* and other coliform bacteria on Rapid *E. coli* Agar (Bio Rad, Finland) at 37 °C for 24 hours, micromycetes on Chloramphenicol Glucose Yeast Extract Agar (Biokar Diagnostics, France) at 25 °C for 120 hours, and *Enterococcus* bacteria on Slanetz-Bartley Agar (Merck, Germany) at 37 °C for 72 hours. After incubation, the number of typical colonies were counted and the results were expressed in CFU/g of the sample and adjusted for the needs of the article on log CFU/g.

RESULTS

For stored the insect dry powder after the first 2 months of storage, the TCM increased in samples stored in the plastic sampler, both in the cold and at the laboratory temperature, from the original values of 1 log CFU/g to 1.663 log CFU/g for the cold stored sample and 2.260 log CFU/g for a sample stored at room temperature.

After the next three months, the sample stored at the plastic container at room temperature decreased to 1.362 log CFU/g TCM. For a sample stored in the sample container and in the cold, the TCM remained the same. The highest increase in TCM occurred in a bag stored in a paper bag and in the cold at 2.870 log CFU/g. For other samples, the TCM ranged in units or tens of CFU/g.

Coliform bacteria were detected in the bag stored in the cold in an amount of 1.839 log CFU/g. Also, thermoresistant microorganisms were also found. The numbers ranged in units to tens of CFU/g. Samples stored in vacuum packs were analyzed only after 5 and 8 months of storage. In this method of storage after 5 months were detected only TCM values 0.954 log CFU/g for the sample stored at 22–23 °C and 0.903 log CFU/g for the sample stored at 4–6 °C. Thermoresistant microorganisms were detected in all samples observed except for the sample stored in the vacuum pack at 4–6 °C. At the last analysis after eight months of storage, molds were detected in all samples except samples stored in the plastic sampler and vacuum packs at 22–23 °C. Other monitored groups of microorganisms were not detected in the samples. Detailed results are given in Tab. I.

A basic chemical analysis of the insect dry powder was performed in the experiment. The analysis revealed that the insect dry powder contained 95.14 g/100 g of dry matter, 2.81 g/100 g of ash, 40.10 g/100 g of nitrogenous substances and 44.83 g/100 g of fat.

DISCUSSION

Klunder *et al.* (2012) investigated the microbiology of edible insects and the influence of different heat treatments on its microbiology. The total number of microorganisms was observed in *Tenebrio molitor*. The values obtained were 1.7 log CFU/g for whole bodies and 2.5 log CFU/g for crushed insects boiled for 10 minutes. When comparing these values with our results, these are very high values, which we are approaching in our experiment after several months of storage.

Caparros Megido *et al.* (2017) dealt with microbiology of edible insects. For *Tenebrio molitor*, lyophilization was used instead of hot air drying. The total number of microorganisms, yeasts and fungi were determined. The value of the total number of microorganisms was 4.47 log CFU/g and the yeast and mold value was less than 1 log CFU/g. When compared to our results, the TCM values are very high, which is, in our opinion, due to the chosen method of sample modification.

Garofalo *et al.* (2017), in his work, dealt with the microbiology of dried *Tenebrio molitor*. The authors do not mention the drying parameters because already dried samples were purchased. He monitored the total number of microorganisms, yeasts and molds. For all observed groups of microorganisms, except mold, he found values less than 2 log CFU/g. For molds, the value was 2.21 log CFU/g.

I: *Results of microbiological analysis of the insect dry powder stored at different conditions (log CFU/g)*

	Coliforms	TCM	Enterococci	TM	Yeasts	Molds
11. 7. 2017						
Worm powder – starting material	ND	1	ND	ND	ND	0.699
18. 9. 2017						
Worm powder – plastic sampler 22–23 °C	ND	2.260	ND	0.699	ND	1.279
Worm powder – paper bag 22–23 °C	ND	ND	ND	1.146	ND	ND
Worm powder – vacuum 22–23 °C	analysis was not performed					
Worm powder – plastic sampler 4–6 °C	ND	1.663	ND	ND	ND	1.447
Worm powder – paper bag 4–6 °C	ND	ND	ND	1	ND	0.699
Worm powder – vacuum 4–6 °C	analysis was not performed					
19. 12. 2017						
Worm powder – plastic sampler 22–23 °C	ND	1.362	ND	0.699	ND	ND
Worm powder – paper bag 22–23 °C	ND	1.279	ND	ND	ND	ND
Worm powder – vacuum 22–23 °C	ND	0.954	ND	ND	ND	ND
Worm powder – plastic sampler 4–6 °C	ND	1.663	ND	1.146	K	K
Worm powder – paper bag 4–6 °C	1.839	2.870	ND	1	ND	ND
Worm powder – vacuum 4–6 °C	ND	0.903	ND	ND	ND	ND
21. 3. 2017						
Worm powder – plastic sampler 22–23 °C	ND	0.699	ND	0.699	ND	ND
Worm powder – paper bag 22–23 °C	ND	0.699	ND	0.954	ND	0.699
Worm powder – vacuum 22–23 °C	ND	ND	ND	0.699	ND	ND
Worm powder – plastic sampler 4–6 °C	ND	1.505	ND	0.699	ND	1.431
Worm powder – paper bag 4–6 °C	ND	0.699	ND	0.699	ND	0.699
Worm powder – vacuum 4–6 °C	ND	ND	ND	ND	ND	0.699

Legend: TCM – total count of microorganisms; TM – thermoresistant microorganisms, ND – not detected

Stoops *et al.* (2017) dealt with the microbiology of minced meat produced from *Tenebrio molitor* during storage. The minced meat was composed of mealworm, binding agent and mixture of spice (salt, white pepper, onion powder, nutmeg, paprika powder). He monitored TCM and thermoresistant microorganisms. Samples were stewed for 5 minutes in the pan and stored for 35 days at 3 °C. Upon commencing storage, the TCM values were 2.3 log CFU/g and the number of thermoresistant microorganisms was 1.0 log CFU/g. During storage, the TCM increased to 7.6 log CFU/g and thermoresistant microorganisms to 2.1 log CFU/g. When compared to our results, these are high values that can be caused by the due to the chosen method of sample preparation.

Osimani *et al.* (2018) dealt with microbiology of the insect dry powder made from crickets of the species *Acheta domesticus*. The number of yeasts and thermoresistant bacteria were determined. The number of yeasts was determined to be less than 1 log CFU/g, spore-forming bacteria 5.52 log CFU/g. When compared to our results, these values are values that we did not achieve even after 8 months of storage. Garofalo *et al.* (2017) also studied the same species of crickets. TCM counts were 4.8 log CFU/g, yeast less than 2 log CFU/g and mold 2.92 log CFU/g. Compared to our results, they are again high values. This difference can be due to chosen method of production of the tested material and insect species.

CONCLUSION

The results of our research show that the microbiologically tested the insect dry powder is a very stable material. After eight months of storage, there was only a minimal increase in count of microorganisms. Based on the results, it is not possible to decide clearly which storage conditions are most appropriate. In our opinion, storage in a paper bag at a temperature of 22–23 °C is considered to

be very convenient, whereas storage at 4–6 °C and 90% humidity cannot be recommended because of the higher incidence of mold due to high humidity in the refrigerator. Due to the small number of expert publications dealing with this issue, it is necessary to continue to address this topic.

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