

## Edible earthworms in a food safety perspective: Preliminary data

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### Abstract

The world population and global food demand are increasing, particularly the demand for animal protein sources. At the same time, society produces large quantities of food waste. Sustainable solutions, to ensure enough food and to optimize the use of resources, are necessary. Earthworms grown on fruit and vegetable waste (FVW) can be a future alternative food source, contributing to waste disposal efficiency. They improve food sustainability under nutritional and environmental dimensions. These topics are included in the philosophy of the circular economy. Earthworms, characterized by a high percentage of proteins and minerals, are used as foods in some world countries, including China and the Philippines. In order to consider safety aspects of earthworms grown on FVW as food sources, this study evaluated the microbiological quality of FVW (i) used as growth substrate; fresh earthworms (ii) and earthworms' meal (iii) resulting from two technological processes (freeze-drying and drying). The efficiency of these technologies in reducing microbial contamination was evaluated. Microbiological analyses revealed the absence of *Salmonella* spp. and *Listeria monocytogenes* in FVW, in fresh earthworms and in earthworms' meal. Fresh earthworms' results fell within the limits of acceptability, if related to the limit for minced meat (Interdepartmental Center for Research and Documentation on Food Safety). Both freeze-drying and drying step led to a further reduction of microbial contamination, confirming the importance of the processing methods. In conclusion, earthworms can represent an innovative biotechnological response to re-use FVW, a valuable food supplement of animal proteins and a strategy to improve food sustainability.

### Introduction

The world population and global food demand are increasing, particularly the demand for animal protein sources, which are the most limiting and expensive in terms of resources (Alexandratos and Bruinsma, 2012; United Nations, 2017). At the same time, society produces large quantities of food waste. Globally 1.3 billion tons of food produced for human consumption is lost or wasted yearly. In particular, the fruit and vegetable sector generates large amounts of waste, especially in industrialized regions (FAO, 2011). All these food losses and waste bring to the squandering of economic, social and environmental resources that have been used to produce food uselessly. Considering the nutritional value, a lot of food ends its "life" with high nutritive elements in it. Finding sustainable and successful strategies against food wastage is necessary and a priority. These topics are included in the philosophy of the circular economy (European Commission, 2015).

The introduction of new foodstuffs is likely to be the right approach for sustainable living.

Earthworms grown on fruit and vegetable waste (FVW) contribute to waste disposal efficiency and are an interesting solution because transform FVW into valuable products: the vermicomposting, that can be sold as organic fertilizer, and the earthworms themselves that, thanks to their high protein content, can be a new food source. Earthworms are eaten in some areas of the world, including China and the Philippines (IFIS, 2009). Based on their nutrient content, earthworms *Eisenia foetida* are an excellent source of readily available protein and minerals in human diet and are included in the Dictionary of Food Science and Technology (IFIS, 2009). Several researches underlined the nutritional values of earthworms as food source (Anitha and Jayraaj, 2012; Cayot *et al.*, 2009; Marconi *et al.*, 2002; Paoletti *et al.*, 2003; Sabine, 1983; Zhenjun and Jiang, 2017). According to the literature data, earthworms *Eisenia foetida* meal has high protein content in the range of 55 to 70% dry matter (Cayot *et al.*, 2009; Edwards, 1985; Medina *et al.*, 2003; Zhenjun *et al.*, 1997; Zhenjun, 2005) and they are rich in the amino acids considered essential for humans (Zhenjun and Jiang, 2017).

Despite research on nutritional values of earthworms as food, the information concerning their food safety aspects remains limited.

In this study, an evaluation of microbial content of fresh and processed earthworms

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was conducted. The aim of the study was to evaluate the microbiological safety of FVW (i) used as growth substrate, fresh earthworms (ii) and earthworms meal (iii) resulting from two technological transformation processes (freeze-drying and drying). Furthermore, the efficiency of these technologies in reducing microbial contamination was evaluated.

### Materials and Methods

#### FVW and earthworms samples

Earthworms from the species of *Eisenia foetida* were reared in a farm located in the province of Lecco (Italy). The production system was an area of 34 m<sup>2</sup>. Growth substrate consisted of FVW and straw. FVW were provided by a fruit and vegetable producer of ready-to-eat products. In order to guarantee optimum growth conditions, the moisture, temperature and pH of the growth substrate were kept under control. The experiment lasted 3 months.

Samples of FVW and fresh earthworms *Eisenia foetida* for microbiological analyses

were collected at the beginning of the rearing process (T1), in the middle (T2) at the end of it (T3).

### Cleaning procedure

The first cleaning procedure consisted of a mechanically separation of earthworms from the growth substrate with the use of a trommel. Afterwards, they were repeatedly washed with running tap water and soaked, in order to remove the residual particles of waste and to clear their gut. Finally, after the excess water was removed with tissue paper, earthworms were packaged in plastic bags, weighed and stored at -28°C.

### Technological transformation processes

Dry meal resulted from two technological transformation processes: freeze-drying and drying. In the first one, the samples were freeze-dried and ground with a

mechanical crusher. In the second, dry meal was produced by drying them in an oven at 50°C and grinding. The two obtained dry meal were placed in vacuum plastic bags and stored at -28°C until the subsequent analyses.

### Microbiological analyses

The analyses focused on the microbiological quality of FVW growth substrate, fresh earthworms and earthworms meal, following the experimental design reported in Figure 1.

Considering that the microbiological content of earthworms depends on the growth substrate, for both FVW and earthworms samples the following parameters were investigated: *Salmonella* spp., *Listeria monocytogenes*, mesophilic aerobic bacteria, *Enterobacteriaceae*, *E. coli*, total coliforms bacteria, coagulase-positive

*Staphylococci*, *Bacillus cereus* and sulphite-reducing clostridia.

An analytical unit (10 g) was aseptically taken from each sample, added to 90 mL of sterile solution (0.85% NaCl and 0.1% peptone), and homogenized in a stomacher and then serial 10-fold dilutions were prepared in a sterile saline solution. *Salmonella* spp. detection (analytical unit 25 g) was carried out using UNI EN ISO 6579:2008 (ISO, 2008). The detection of *L. monocytogenes* (analytical unit 25 g) was performed according to AFNOR BRD 07/4-09/98. Mesophilic aerobic bacteria and *Enterobacteriaceae* were enumerated using a Petrifilm (3M, St. Paul, Minnesota, USA), following the AFNOR 3M 01/1-09/89 and AFNOR 3M 01/06-09/97, respectively. Petrifilm plates were also used to determine *E. coli*, total coliforms bacteria and coagulase-positive *Staphylococci* in accordance

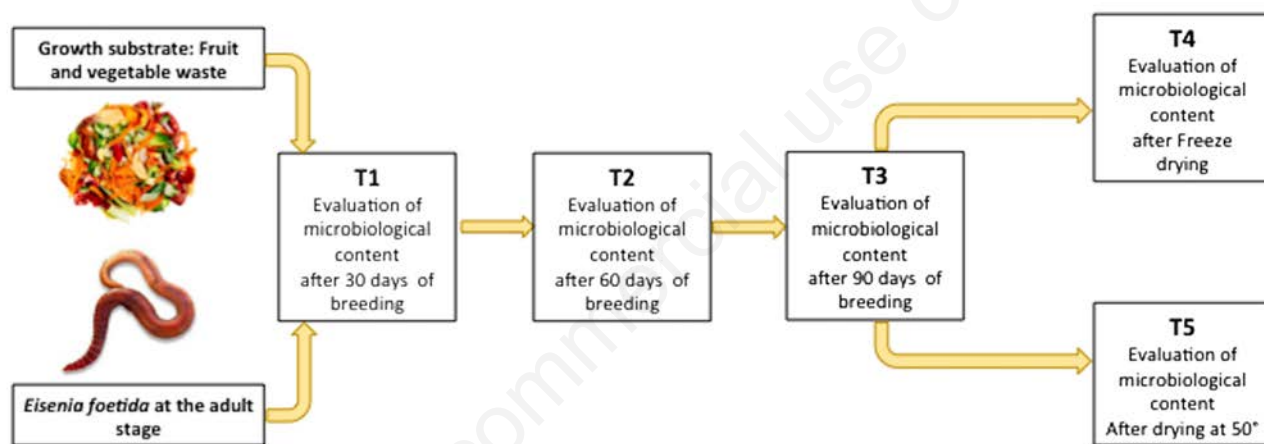


Figure 1. Experimental design.

Table 1. Standard CeIRSA guidelines for unprocessed raw fresh fruits and vegetables.

Bacteriological parameters	Standard Ce.I.R.S.A guidelines (CFU/g)		
	Satisfying	Acceptable	Unsatisfying
<i>Listeria monocytogenes</i>	Absent in 25 g	-	Presence in 25 g
<i>Salmonella</i> spp.	Absent in 25 g	-	Presence in 25 g

Table 2. Standard CeIRSA guidelines for minced meat.

Bacteriological parameters	Ce.I.R.S.A guidelines (CFU/g)		
	Satisfying	Acceptable	Unsatisfying
<i>Listeria monocytogenes</i>	Absent in 25 g		Presence in 25 g
<i>Salmonella</i> spp.	Absent in 25 g		Presence in 25 g
Mesophilic aerobic bacteria	$<5 \times 10^5$	$5 \times 10^5 \leq x < 5 \times 10^6$	$\geq 5 \times 10^6$
<i>Enterobacteriaceae</i>	$< 10^2$	$10^2 \leq x < 10^3$	$\geq 10^3$
<i>E. coli</i>	$< 5 \times 10$	$5 \times 10 \leq x < 10^2$	$\geq 5 \times 10^2$
Sulphite-reducing clostridia	$< 10^2$	$< 10^2 \leq x < 10^3$	$\geq 10^3$

with the following methods: AFNOR 3M 01/08-06/01, AFNOR 3M 01/2-09/89 and AFNOR 3M 01/ 9-04/03, respectively. *Bacillus cereus* count was enumerated according to UNI EN ISO 7932:2005 (UNI, 2005). The sulphite-reducing clostridia were determined on iron sulphite agar after incubation in aerobic atmosphere at 37°C ±1 for 24-48 hours.

### Safety and hygiene criteria

The results were compared with the guidelines provided by Interdepartmental Center for Research and Documentation on Food Safety (CeIRSA). In particular, the results obtained from FVW samples were compared with the limits provided for unprocessed raw fresh fruits and vegetables category (Table 1), while fresh and processed earthworms results were compared to minced meat category (Table 2).

## Results

### Growth substrate

Microbiological content of FVW revealed the absence of *Salmonella* spp. and *Listeria monocytogenes* in all samples. The other microbiological parameters evaluated for FVW are shown in Table 3.

**Table 3. Microbiological results of FVW growth substrate.**

Bacteriological parameters	Microbial counts (CFU/g) <sup>a</sup>		
	T1 (30 d)	T2 (60 d)	T3 (90 d)
Mesophilic aerobic bacteria	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>5</sup> -10 <sup>6</sup>
<i>Enterobacteriaceae</i>	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>3</sup> -10 <sup>4</sup>
<i>E. coli</i>	10 <sup>3</sup> -10 <sup>4</sup>	10 <sup>3</sup> -10 <sup>4</sup>	<10
Coliforms bacteria	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>5</sup> -10 <sup>6</sup>
Coagulase-positive <i>Staphylococci</i>	<10	<10	<10
<i>Bacillus cereus</i>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>5</sup> -10 <sup>6</sup>
Sulphite-reducing clostridia	10 <sup>3</sup> -10 <sup>4</sup>	10 <sup>2</sup> -10 <sup>3</sup>	<10

<sup>a</sup>The results are expressed as range.

**Table 4. Microbiological results of fresh earthworms.**

Bacteriological parameters	Microbial counts (CFU/g) <sup>a</sup>		
	T1 (30 d)	T2 (60 d)	T3 (90 d)
Mesophilic aerobic bacteria	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>4</sup> -10 <sup>5</sup>
<i>Enterobacteriaceae</i>	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>3</sup> -10 <sup>4</sup>
<i>E. coli</i>	10 <sup>3</sup> -10 <sup>4</sup>	<10	<10
Coliforms bacteria	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>4</sup> -10 <sup>5</sup>	10 <sup>3</sup> -10 <sup>4</sup>
Coagulase-positive <i>Staphylococci</i>	<10	<10	<10
<i>Bacillus cereus</i>	10 <sup>3</sup> -10 <sup>4</sup>	10 <sup>2</sup> -10 <sup>3</sup>	10 <sup>2</sup> -10 <sup>3</sup>
Sulphite reducing clostridia	10 <sup>3</sup> -10 <sup>4</sup>	10 <sup>3</sup> -10 <sup>4</sup>	<10

<sup>a</sup>The results are expressed as range.

### Fresh earthworms and earthworms meal

Microbiological content of fresh earthworms and earthworms meal (freeze-drying and drying) revealed the absence of *Salmonella* spp. and *Listeria monocytogenes* in all samples, conforming to the Regulation (EU) 2073/2005 on microbiological criteria for foodstuffs (European Commission, 2005). The results of the remaining microbiological parameters evaluated for fresh earthworms are shown in Table 4. In earthworms the microbiological contamination was reduced by the use of the two different technological drying processes for meal production.

### Discussion

In compliance with the Regulation EC No 2073/2005 (European Commission, 2005), no *Salmonella* spp. and *L. monocytogenes* were detected in FVW (growth substrate), fresh earthworms and earthworms processed meal samples. The absence of *Salmonella* spp. and *L. monocytogenes* is a criterion for food safety.

The FVW used as growth substrate were comparable to CeIRSA standards for unprocessed raw fresh fruits and vegetables category (CeIRSA, 2013).

Fresh earthworms resulted acceptable

for all the parameters considered, except for *Enterobacteriaceae*, concerning CeIRSA minced meat category. However, the application of freeze-drying (T4) and drying (T5) processes to earthworms showed a reduction of all microbial parameters considered by CeIRSA, and were comparable to satisfying category reported for minced meat (CeIRSA, 2013). This confirmed the importance of the treatments to reduce the bacterial contamination in different terrestrial invertebrates proposed as food, as reported by Caparros Megido *et al.* (2017) and Grabowsky *et al.* (2017).

### Conclusions

Possible food safety hazards associated with the use of novel proteins in food applications need to be prevented, following the European Union rules for animal deriving products. These hazards are associated to the rearing conditions (feed and environment) and the subsequent food technological process.

This study presented a general assessment of the microbiological evaluation on earthworms if they will be reared for food purposes. The research evidenced that earthworms meal could be a safe food for microbiological criteria. Furthermore, the results underlined the importance of the

processing methods (freeze-drying and drying) in the reduction of microbial contamination. As in other edible terrestrial invertebrates production, proper technological processing, packaging and storage conditions need to be considered in order to prevent the microbial contamination.

The next step to ensure safer products for consumers should be the establishment of specific guidelines for the production and commercialization of earthworms if they will be reared for human consumption.

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