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Review

Bombyx mori from a food safety perspective: A systematic review

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ABSTRACT

Bombyx mori (BM) is an economically important insect for silk production, and it is also farmed and used as food in different countries around the world. The present systematic review aims to assess the suitability of BM as an edible insect, retrieving data from scientific papers reporting microbiological, chemical, and allergenic hazards of silkworm consumed as either whole insects or insect derivatives. We considered all studies published in peer-reviewed journals in English, French, and Spanish languages. No time limits were imposed. We searched PUBMED, WEB of Science Core Collection, and EMBASE databases. The last literature search was carried out on May 5th, 2021. Data were collected in pre-defined tabular forms for the aforementioned hazards. In total, 65 records investigating the safety aspects were included after screening: 32 on microbiological hazards; 27 on chemical hazards; 16 on allergenic hazards. Concerning microbiological aspects, a high presence of *Enterococcus* in raw insects (5.00 % to 70.10 %) was reported through metagenomic analysis. Through non-metagenomic methods (classical and biomolecular microbiology techniques), *Bacillus cereus* and *Pseudomonas fluorescens* were the most commonly investigated and detected bacteria in the unprocessed insects, while *B. cereus* and *Enterobacteriaceae* were studied and reported in insect-based food. The foodborne pathogens *Listeria monocytogenes* and *Salmonella* spp. were never detected.

Concerning toxicological aspects, three studies assessed the toxicity of BM powder in laboratory animals, but no negative effects were observed. Regarding heavy metal bioaccumulation in BM, evidence was reported for As, Cu, and, Zn.

Allergic reactions following the ingestion of BM or derivative products are due to proteins that are widespread in arthropods. Furthermore, BM proteins can undergo possible cross-reactions with proteins of other insect species or crustaceans. However, heat treatments do not seem to reduce the allergenic potential of the silkworm proteins.

The major limitation of the present review is that we could include only scientific literature published in Western languages, while the majority of relevant studies were conducted in Asian countries and part of them are published in Asian languages.

In conclusion, scientific evidence regarding microbiological and chemical hazards of BM relevant for food safety is very limited. In the present work microbiological and chemical hazards relevant for food safety were identified in BM, however their presence do not impair the use as food but suggest the need for a risk assessment under specific conditions of production and use. Allergic reactions are possible in sensitive individuals following the ingestion of edible BM.

1. Introduction

The use of insects as food or as ingredients for food and feed is arousing interest worldwide. Globally, >1,900 insect species have reportedly been used as food, especially in developing countries (FAO, 2013). Advantages of insect farming are related to the high

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environmental sustainability, efficient use of land and water resources, as well as decreased greenhouse gas emissions, compared to intensive livestock breeding (Hayes et al., 2013). Moreover, insects are a valuable nutritive source of high value proteins and fats (Osimani et al., 2017), minerals, vitamins, and fiber due to the presence of chitin (Belluco et al., 2013).

However, the food safety risks deriving from insect consumption need further investigation, and in particular, need assessment by species. Microbial hazards depend on both the gut microbiota composition of insects, since they are processed both as food and as feed with their intestinal content, and possible contamination with pathogens. They depend on the nature and the hygienic conditions of the rearing substrate and the farming environment (EFSA, 2015). Chemical hazards encompass environmental contaminants, e.g. heavy metals (Green et al., 2001; Handley et al., 2007; Lindqvist, 1992; Merrington et al., 1997; Vijver et al., 2003; Zhuang et al., 2009), dioxins (Devkota & Schmidt, 2000; Hunter et al., 1987; Jamil & Hussain, 1992), polybrominated diphenyl ethers (Gaylor et al., 2012), mycotoxins, and plant toxins, which may be present in the rearing substrates for insects. In addition, toxins produced by insects should be considered as chemical hazards. One advantage regarding the above-mentioned hazards is that edible insects can be farmed under controlled conditions (Hanboonsong et al., 2013). The hazard of individuals developing allergic reactions following insect consumption is another important issue to be taken into account given the high protein content of insects. Allergic reaction and anaphylactic shock in humans have been documented after insect ingestion (Fao, 2013; Ji et al., 2009).

The European Regulation categorizes insect-based food as *novel food* according to Reg. 2015/2283 (European Commission (EU), 2015). In the first European Food Safety Authority (EFSA) opinion on the risk profile of edible insects, which followed previous opinions of national food safety authorities, a starting list of 12 species of insects was considered, including the silkworm, *Bombyx mori* (BM) (EFSA, 2015). Based on the positive scientific opinion of EFSA, the European Commission has already authorized the trade of some processed insects, and some other species are under evaluation.

BM is an herbivore that feeds on mulberry (*Morus* sp.*pl.*) leaves; it is an economically important insect that has been domesticated for thousands of years, mainly in Asia, to maximize silk fiber productivity (Xia et al., 2004; Yang et al., 2014). Besides a mulberry leaf-based diet, an artificial diet has also been developed for sericulture (Bhattacharyya et al., 2016). To date, BM is already traditionally farmed and used as food in Central America (Mexico) and in Asia (Thailand, Myanmar, Vietnam, India, Laos, China, Japan, and Korea). The present document aims to review all the available evidence on BM from a food safety perspective. Scientific papers reporting microbiological, chemical, toxicological, and allergological data concerning BM as reared insects, processed food and/or feed were included and summarized. The review question was: "Which are the hazards of BM relevant for food safety?".

2. Materials and methods

2.1. Eligibility criteria, information sources, and search strategies

We considered all studies published in peer-reviewed journals in English, French, and Spanish languages. No time limits were imposed. On May 14th, 2020, we searched PUBMED, WEB of Science Core Collection, and EMBASE (Title/Abstract, Topic (TS) and Title, Abstract, Author keywords, respectively) with the search terms reported in Table 1. An update of the search was conducted on May 5th, 2021.

The search strategy of the present work included terms also referring to the nutritional aspects and the chemical composition of BM (Table 1); records referring to this second topic (nutritional aspects/chemical composition) were identified during the initial screening and used for another systematic review. To implement the search process, we used the final list of studies to carry out a backward reference search in order to identify potential missing evidence.

Table 1
Keywords employed to retrieve relevant records reporting data on BM food safety and composition.

Keywords (Title/Abstract)
Bombyx OR Silkworm OR "silk worms" OR silkmoth OR "silk moths"
AND
nutrition OR composition OR centesimal OR nutrient OR nutrients OR protein OR proteins peptide OR peptides OR aminoacid OR aminoacids OR "amino acid" OR "amino acids" OR acid OR acids OR polypeptide OR polypeptides OR fat OR fats OR lipid OR lipids OR "fatty acid" OR "fatty acids" OR "fatty alcohols" OR sugar OR sugars OR carbohydrate OR carbohydrates OR disaccharide OR disaccharides OR monosaccharide OR monosaccharides OR polysaccharide OR polysaccharides OR ash OR mineral OR minerals OR macronutrient OR macronutrients OR micronutrient OR micronutrients OR oligoelement OR oligoelements OR microelement OR microelements OR vitamin OR vitamins OR oil OR oils OR "trace element" OR "trace elements"
OR
safety OR hazard OR hazards OR risk OR risks OR microorganism OR microorganisms OR pathogen OR pathogens OR contaminant OR contaminants OR contamination OR contaminations OR chemical OR chemicals OR toxic OR toxics OR toxicity OR metal OR metals OR toxin OR toxins OR allergy OR allergies OR allergen OR allergens OR allergic OR allergenic OR sensitization OR sensitisation OR cross-reactivity OR anaphylactic OR anaphylaxis OR poisoning OR poison OR compound OR compounds OR pesticide OR pesticides OR residual OR residue OR residues OR antibiotic OR antibiotics OR antiparasitic OR antiparasitics OR mycotoxin OR mycotoxins OR dioxin OR dioxins OR polluting OR pollutant OR pollutants

Several criteria were used to select eligible studies: (1) the study had to be in English, Spanish, or French; (2) reported data had to belong to primary research and not to other reviews; (3) the study had to deal with microbial, chemical, or allergen hazards relevant to BM consumption.

2.2. Selection process

The screening process was carried out using the EPPI-4 Reviewer software (Thomas et al., 2010). The first classification was carried out by six reviewers (SB, FM, AP, LT, AS, SC) in order to include or exclude papers based on the topic. Thereafter, five reviewers (SB, FM, PA, LT, AS) categorized all studies obtained via the initial literature search into nutritional composition and microbiological, chemical, toxicological or allergenic hazard based on Title, Abstract. In the case of a poorly explicative abstract or in the case of doubt about the available data, the study was included and evaluated at full-text level. Disagreements were resolved through consensus. All studies were coded according to the previously chosen parameters, and data were recorded.

Data from the included studies were entered into pre-defined tabular forms, one for each of the hazard categories. Common variables useful to describe the study design were retrieved: country, life cycle stadium, sample origin, diet, and analytical method.

It was not possible to assess the study-level risk of bias because of differences in the study design and high variability of the methodology employed in the included studies. According to the specificity of the studies referring to different topics, additional forms were used to extract data.

2.3. Data items and data collection process

2.3.1. Data items and collection process for microbial hazards

In the microbial hazards section we defined "study" as those investigations that employed metagenomic (MG) analysis and those that employed non-metagenomic (NMG) analysis such as classical and biomolecular microbiology techniques. General data related to included studies were listed in tables reporting the following information: *i)* year of the study; *ii)* country where the study was carried out; *iii)* sample origin; *iv)* BM life stage; *v)* type of sample (unprocessed insect or insect-based food); *vi)* insect diet, and; *vii)* analytical technique description.

The data collected for NMG studies were listed in tables reporting the

detected genera/species of microorganisms. The data collected for insect-based food were listed in tables reporting the following items: *i)* detected genera/species; *ii)* ratio between the studies which confirmed the presence of that genera/species: studies that intended to detect that genera/species, and; *iii)* type of insect-based food. Also, for NMG analysis, data concerning bacteria counts were reported in tables that indicated the type of target analysis, the reported bacteria count, and additional information concerning tested samples.

The data concerning MG analysis for insect-based food and unprocessed insects were collected in a table reporting data on BM characteristics: diet; life cycle stage; strain, and; the related bacterial genera detected.

2.3.2. Data items and collection process for chemical hazards

In the section dealing with chemical hazards we defined "study" as an investigation carried out on a specific target with an "ad hoc" study design. Data collected from studies investigating chemical hazards were different according to each study aim. From studies dealing with toxicity we collected data about: *i)* insect species (strain); *ii)* diet/feed; *iii)* life cycle stage; *iv)* endpoint; *v)* in vitro toxicity target; *vi)* in vivo toxicity target; *vii)* analytical methods; *viii)* matrix tested; *ix)* target animal; *x)* number of laboratory animals used; *xi)* doses; *xii)* acute toxicity doses tested; *xiii)* duration of experiments; *xiv)* time points, and; *xv)* results. From studies dealing with chemical contamination, we collected data about: (*i)* insect species; (*ii)* sample origin; (*iii)* diet/feed; (*iv)* life cycle stage; (*v)* origin of contamination; (*vi)* hazard; (*vii)* methods; (*viii)* exposure; (*ix)* soil; (*x)* leaves (dry weight); (*xi)* silkworm (dry weight) and feces (dry weight) contamination level; (*xii)* soil-leaf; (*xiii)* leaf-silkworm, and; (*xiv)* silkworm-feces transfer factor.

2.3.3. Data items and collection process for allergenic hazards

Studies investigating the allergenic hazards related to BM were categorized in three different sets: (*i)* studies reporting people with allergic symptomatology following the ingestion of BM; (*ii)* studies that provided proteomic characterization of potential allergenic determinants of BM, along with the investigation of possible cross-reactivity and similarity with other proteins isolated from other organisms, and; (*iii)* studies investigating the effects of heat on the allergenic potential of BM. Data from each set were synthesized, in tables, as follows: *set* (*i*) whether or not the allergic reaction to BM in the patients was confirmed through laboratory analysis and the resulting symptomatology; *set* (*ii*) methods used for protein characterization, molecular weight of the identified proteins and, where mentioned, their name. Finally, if there were possible correlations or similarities between BM proteins with those from other organisms; *set* (*iii*) the BM matrix subjected to the heat treatment, along with time and temperature of treatment. Then, which methods were used to evaluate the effects of treatment on the allergenicity of BM. Last, the results of treatment on BM, as reported in the study.

Along with the above-mentioned data, in all tables, data on BM life stage and the country of study were collected. More specifically, for those studies reporting cases of allergic symptomatology, data on the number of patients involved were also collected.

2.4. Synthesis

2.4.1. Synthesis of microbial hazards

Concerning the microbial hazards, for NMG studies, the frequency of a given microorganism as reported was extracted, whereas for MG studies, data were collected on those bacteria genera with a relative abundance ≥ 5 % with respect to the genera detected in each study. The extracted data were included in tables that differentiated unprocessed insects from the insect-based food.

2.4.2. Synthesis of chemical hazards

The data on studies dealing with chemical contaminants were extracted in more data tables designed to capture the high heterogeneity of the study designs. In particular, a general table was built to describe relevant information about general characteristics of the included studies, and other tables were populated with the study results according to the study aim (toxicity vs contamination) and to the target contaminant.

2.4.3. Synthesis of allergenic hazards

Regarding the allergenic hazards of BM, following identification of the eligible studies, a further categorization was applied according to the information therein reported. In particular, data on the characterization of potentially allergenic determinants, on case reports of patients with allergic reaction following the ingestion of BM, and on the effects of thermal treatments on BM allergenicity were extracted and included in tables.

3. Results

In total, 65 records investigating food safety aspects related to BM were included after screening (Fig. 1). In particular, 32 records concerned the microbial hazards, 17 the chemical hazards, and 16 the allergenic hazards. The 156 records regarding nutritional aspects underwent another systematic review.

3.1. Microbial hazards associated with Bombyx mori consumption

3.1.1. Study characteristics

In total, 32 papers investigated the silkworm BM from a microbiological perspective. The 32 papers included 25 studies performed with NMG techniques (Table 2A) and 12 studies performed with MG techniques (Table 2B).

Six and 31 studies investigated the microbiology of silkworm-based food and raw insects, respectively. One MG and five NMG studies were conducted on silkworm-based food samples. Concerning the geographic area where the studies were performed, Asia was the main location with 30 studies, while one and six studies were conducted in Africa and Europe, respectively. The most investigated BM life stage was the larval phase with 29 studies, while the pupal stage was investigated in four studies concerning food. Four studies did not report any information about the life stage. Regarding the analytic methods, 20 and seven papers employed NMG and MG techniques, respectively, while 5 papers reported both methods. General information concerning the included studies is reported in Table 2.

3.1.2. Results of individual studies

Among the studies included as NMG methods, 20 studies were conducted on insects and 5 studies on silkworm-based food. The bacterial taxa detected in the studies are shown in Tables 3 and 4.

In unprocessed insects, in total 29 bacteria genera and 32 species were detected. The species reported in more than one study were *Bacillus cereus* (4), *Bacillus subtilis* (3), *Citrobacter freundii* (2), *Enterococcus mundtii* (3), *Escherichia coli* (2), *Klebsiella cloacae* (2, only in diseased silkworms), *Pseudomonas fluorescens* (4), *Serratia marcescens* (2), and *Staphylococcus aureus* (2). Also, two fungus genera (*Alternaria* and *Perussia*) and one fungus species (*Coprinellus radians*) were identified in unprocessed insects by one study (Liang et al., 2015).

Concerning the silkworm-based food, in total eight bacteria genera and three species were detected. The detected species were *B. cereus*, *Bacillus lehensis*, and *Paenibacillus apiarius*. *B. cereus* was reported by two studies, while in one study it was searched for but not detected. The presence of bacteria belonging to *Enterobacteriaceae* was also investigated by three studies but only two reported their presence.

The presence of foodborne pathogens, *E. coli*, *Listeria monocytogenes*, *Salmonella* spp., *S. aureus*, and Shiga toxin-producing *E. coli* (STEC) was investigated by some studies (Fasolato et al., 2018; Grabowski & Klein, 2017a; Osimani et al., 2018a), but none of these pathogens were

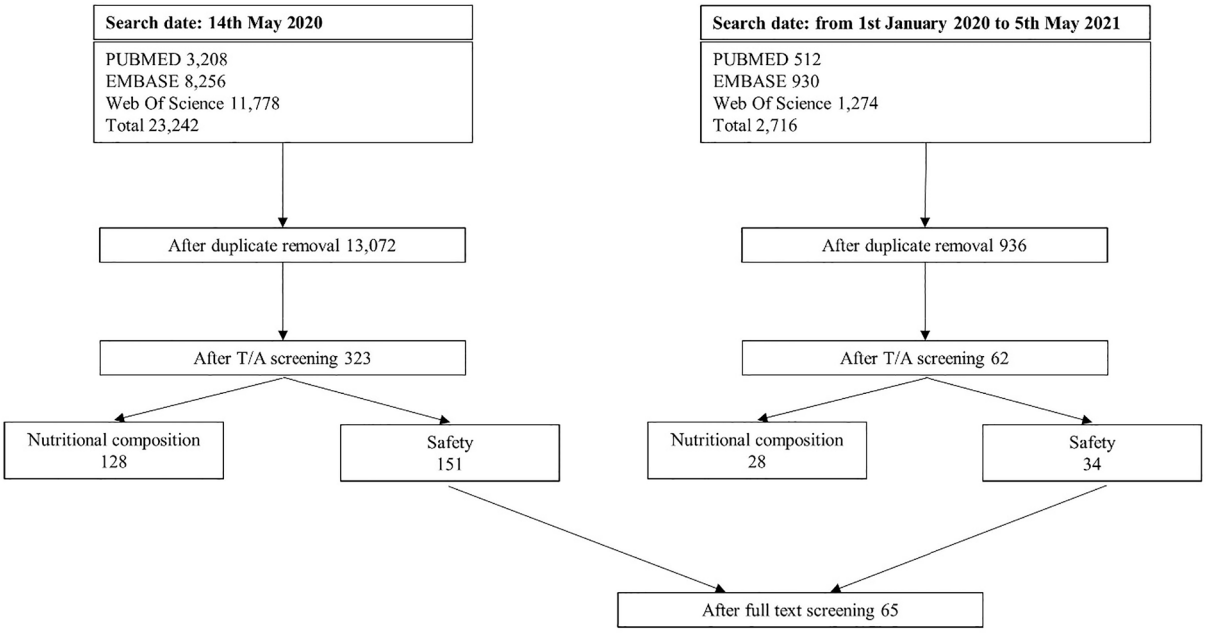

Fig. 1. The flow diagram reports the number of retrieved records from three databases (PUBMED, EMBASE and Web of Science) and after title/abstract (T/A) screening. After full-text screening, 75 records were included. The two flows represent the two dates when database searches were carried out.

detected.

Data reporting bacteria counts in analyzed samples are reported in Table 5. Concerning unprocessed insects, the total microbial count (TMC) ranged from 3.33 × 10^1 CFU/g (in germ free artificial diet) (Cappellozza et al., 2011) to 6.080 × 10^{11} CFU/ml (Prem Anand et al., 2010), while for the silkworm-based food, the TMC ranged from 3.0 × 10^2 CFU/g (Milanović et al., 2016) to 1.34 × 10^8 CFU/g (Kurdi et al., 2021). In the food, the highest bacteria count reported for *Enterobacteriaceae* was 10^7 CFU/g (Grabowski & Klein, 2017a), while for *B. cereus* reported counts were between 10^2 –10^3 CFU/g (Fasolato et al., 2018).

A total of 12 studies reported MG analysis, of which only one involved silkworm-based food (Table 2B). Only two studies carried out MG analysis on both bacterial and fungal communities (Chen et al., 2018a; Liang et al., 2015), while all the other studies performed MG analysis only on the bacterial community. It was not possible to retrieve the exact percentages of the bacteria present from two studies (Li et al., 2016; Liang et al., 2015), and therefore, the data were extracted only from 10 studies and did not include data derived from feed contaminated with pathogens (Sun et al., 2016) or chemicals (Li et al., 2020), or silkworms exposed to high environmental temperature (Sun et al., 2017). The bacteria with a relative abundance ≥ 5 % with respect to the genera detected in each study are listed in Supplementary Table 1. In total, 29 bacteria genera were reported according to this criterion. The most commonly reported genus was *Enterococcus*, since it was found by six studies with a relative abundance ranging from 5.00 % to 70.10 %. The second most reported genus was *Bacillus,* detected by five studies with a relative abundance ranging from 5.02 % to 25.94 % in the whole insects, while in silkworm-based food it was reported with relative abundance > 80 %. *Pseudomonas* was present only in one study with relative abundance ≥ 5 %.

3.1.3. Synthesis of results

Through NMG methods, the most investigated and detected bacteria were *B. cereus* and *P. fluorescens* in unprocessed insects, and *B. cereus* and *Enterobacteriaceae* in insect-based food. In MG analysis, *Pseudomonas* was present only in one study with a relative abundance ≥ 5 %, while the high presence of *Bacillus* spp. was supported also by MG analysis, since it was the second most commonly reported genus with a relative abundance ranging from 5.02 % to 25.94 % in unprocessed insects, and > 80 % in silkworm-based foods. *B. cereus* and *B. subtilis* were the most commonly reported *Bacillus* species. Moreover, the highest counts of *Enterobacteriaceae* and *B. cereus* in silkworm-based foods were 10^7 CFU/g and between 10^2 –10^3 CFU/g, respectively.

The genus *Escherichia* was recorded only by one study using MG analysis, reporting a relative abundance of 7.78 %, while for unprocessed insects, two studies reported the presence of *E. coli.*

S. aureus was only recorded in diseased, unprocessed silkworms, while in MG analysis, three studies reported *Staphylococcus* with a range of relative abundance from 6.66 % to 9.12 %. Concerning *Clostridium*, only one study reported its presence in food with a count of 2.14 × 10^3 CFU/g, while in one study it was searched for but not detected. In MG analysis, the genus *Clostridium* was detected by two studies and its relative abundance ranged from 16.00 % to 28.14 %.

3.2. Chemical hazards associated with B. mori consumption

3.2.1. Study characteristics

Seventeen papers dealing with BM in the context of chemical safety were retrieved. Three studies investigated toxicological aspects related to the consumption of silkworm meal, while twenty studies investigated the presence or possible accumulation of chemical contaminants in silkworm. All studies were carried out in Asian countries. Eighteen studies investigated the accumulation of heavy metals along the soil to silkworm pathway. These studies investigated the potential of the mulberry tree to amend contaminated soils and also the potential consequence of this practice within the silk production system. The experimental design was similar among studies, with mulberry trees exposed to heavy metals via naturally or artificially contaminated soils and then evaluation of the transfer along the soil, tree (leaves), silkworm, feces chain. Two studies had different aims: one investigated the contamination of insects sold online as food (Aydoğan, 2021) and the other studied an episode of histamine intoxication during a public event (Chomchai & Chomchai, 2018). No studies were retrieved that investigated mycotoxins in BM.

3.2.2. Results of individual studies on toxicity

Three studies investigated the toxicity of BM (Table 8) (Heo et al., 2013; L. Jiang et al., 2021; Rattana et al., 2017). Heo and colleagues

Table 2
General information about non-metagenomic (A) and metagenomic (B) studies included for microbiology research on B. mori as unprocessed insect (UI) or insect-based food (IBF).

A)

References	Year	Country	Sample origin	Life stage	Type of sample (unprocessed insect (UI)/ insect-based food (IBF)	Diet	Techniques employed for non-metagenomic
Kalpana et al.	1994	India	India	Larva	UI	Mulberry leaves	Classical microbiology
Fei et al.	2006	China	NR	NR	UI	NR	Classical microbiology / 16S rRNA seq
Priyadharshini et al.	2008	India	India	Larva	UI *	NR	Classical microbiology
Subramanian et al.	2009	India	NR	NR	UI	NR	16S rRNA
Prem Anand et al.	2010	India	India	Larva	UI	Mulberry leaves (UV sterilized)	Classical microbiology
Cappellozza et al.	2011	Italy	Italy	Larva	UI	Non germ-free artificial diet with chloramphenicol Germ-free artificial diet Mulberry leaves	Classical microbiology / fluorescence microscopy analysis/ ARDRA
Feng et al.	2011	China	NR	Larva	UI	Mulberry leaves Tricupsid cudriana leaves from 4th instar	Classical microbiology / 16S rRNA seq
Pradhap & Selvisabhanayakam	2011	India	NR	Larva	UI	Mulberry leaves (unhygienic conditions)	Classical microbiology / 16S rRNA seq
Tao et al.	2011	China	China	Larva	UI *	Mulberry leaves	Classical microbiology / 16S rRNA seq
Sakthivel et al.	2012	India	India	NR	UI	NR	Classical microbiology
Zhang et al.	2013	China	China	Larva	UI *	NR	Classical microbiology / 16S rRNA seq
Ayoade et al.	2014	Nigeria	Nigeria	Larva	UI	NR	Classical microbiology
Liang et al.	2014	China	China	Larva	UI	Mulberry leaves Lettuce from 4th instar	Classical microbiology/ DGGE electrophoresis
Li et al.	2015	China	China	Larva	UI	NR	Classical microbiology / 16S rRNA seq
Liang et al.	2015	China	China	Larva	UI	Mulberry leaves Lettuce from 4th instar	16S rRNA seq/ ITS seq
Mohanta et al.	2015	Bangladesh	Bangladesh	Larva	UI *	NR	Classical microbiology/ 16S rRNA seq
Li et al.	2016	China	China	Larva	UI	Mulberry leaves	Classical microbiology
Milanović et al.	2016	Italy	Thailand	Pupa	IBF	–	Classical microbiology
Grabowski & Klein	2017a	Germany	Asia or EU	Pupa	IBF	–	Classical microbiology
Chen et al.	2018a	China	China	Larva	UI	Mulberry leaves	DGGE electrophoresis
Fasolato et al.	2018	Italy	Europe, Asia	Larva	IBF	NR	Classical microbiology / 16S rRNA seq
Liang et al.	2018	China	China	Larva	UI	Mulberry leaves	Classical microbiology
Osimani et al.	2018a	Italy	Thailand	Pupa	IBF	–	Real-Time PCR/ DGGE electrophoresis/ sequencing
Zhang et al.	2020	China	China	Larva	UI	NR	Classical microbiology / 16S rRNA seq
Kurdi et al.	2021	Thailand	Thailand	NR	IBF	–	Classical microbiology

B)

References	Year	Country	Sample origin	Life stage	Type of sample	Diet	Techniques employed for metagenomic
Liang et al.	2014	China	China	Larva	UI	Mulberry leaves Lettuce from 4th instar	16S rRNA seq
Liang et al.	2015	China	China	Larva	UI	Mulberry leaves Lettuce from 4th instar	16S rRNA seq/ 18S rRNA seq
Li et al.	2016	China	China	Larva	UI	Mulberry leaves	16S rRNA seq
Sun et al.	2016	China	NR	Larva	UI	Mulberry leaves	16S rRNA seq
Sun et al.	2017	China	NR	Larva	UI	Mulberry leaves	16S rRNA seq
Chen et al.	2018a	China	China	Larva	UI	Mulberry leaves	16S rRNA seq/ ITS seq
Chen, et al.	2018b	China	China	Larva	UI	Mulberry leaves	16S rRNA seq/ NGS
Dong et al.	2018	China	NR	Larva	UI	Mulberry leaves Artificial diet	16S rRNA seq
Hou et al.	2018	China	China	Larva	UI	Mulberry leaves	16S rRNA seq
Osimani et al.	2018a	Italy	Thailand	Pupa	IBF	–	16S rRNA seq
Yeruva et al.	2020	India	NR	Larva	UI	Mulberry leaves	16S rRNA seq
Li et al.	2020	China	China	Larva	UI	Mulberry leaves	16S rRNA seq

NR: not reported.
*Diseased silkworms.

applied a series of techniques to characterize the toxicological potential of lyophilized powder obtained from the silkworm larvae. A reverse mutation test with *Salmonella* Typhimurium using doses ranging from 0 to 5,000 μg/plate showed negative results, as did the chromosomal aberration assay (doses up to 1,100 μg/ml), and the bone marrow micronucleus assay (doses up to 5,000 mg/Kg). Acute and subchronic toxicity studies were carried out on Sprague-Dawley rats with, respectively, a single dose up to 5,000 mg/Kg and a repeated dose up to 2,000 mg/Kg/day for 90 days. No signs of toxicity were observed, and thus, >5,000 mg/Kg and 2,000 mg/Kg were set as the lethal dose and "No Observed Adverse Effect Level" (NOAEL) dose, respectively (Heo et al., 2013).

Table 3
List of bacteria genera and/or species detected in B. mori (unprocessed insects) through non-metagenomic analysis.

Bacteria detected in unprocessed insects	References	Bacteria detected in unprocessed insects	References
Acinetobacter spp.	Kalpana et al., 1994	*Klebsiella cloacae*	Priyadharshini et al., 2008; Sakthivel et al., 2012
Aeromonas spp.	Feng et al., 2011; Kalpana et al., 1994; Prem Anand et al., 2010	*Klebsiella granulomatis*	Mohanta et al., 2015
Aeromonas hydrophila	Liang et al., 2014	*Klebsiella pneumoniae*	Prem Anand et al., 2010
Agrobacterium spp.	Feng et al., 2011	*Lysobacter* spp.	Liang et al., 2014
Alcaligenes spp.	Kalpana et al., 1994; Liang et al., 2014	*Microbacterium oxydans*	Liang et al., 2014; Liang et al., 2018
Bacillus spp.	Feng et al., 2011; Kalpana et al., 1994; Subramanian et al., 2009	*Micrococcus* spp.	Fasolato et al., 2018
Bacillus badius	Ayoade et al., 2014	*Moraxellaceae*	Liang et al., 2014
Bacillus cereus	Li et al., 2015; Liang et al., 2018; Priyadharshini et al., 2008; Sakthivel et al., 2012	*Moraxella* spp.	Kalpana et al., 1994
Bacillus circulans	Cappellozza et al., 2011; Prem Anand et al., 2010	*Pantoea* spp.	Liang et al., 2014; Liang et al., 2015
Bacillus licheniformis	Cappellozza et al., 2011	*Pectobacterium carotovorum*	Liang et al., 2014
Bacillus subtilis	Priyadharshini et al., 2008; Sakthivel et al., 2012; Subramanian et al., 2009	*Proteus vulgaris*	Prem Anand et al., 2010
Bacillus thuringiensis	Priyadharshini et al., 2008	*Providencia rettgeri*	Zhang et al., 2013
Brevibacterium spp.	Feng et al., 2011	*Pseudomonas* spp.	Subramanian et al., 2009; Feng et al., 2011
Brevundimonas spp.	Feng et al., 2011	*Pseudomonas aeruginosa*	Prem Anand et al., 2010
Citrobacter spp.	Feng et al., 2011	*Pseudomonas cloraphis*	Tao et al., 2011
Citrobacter amalomaticus	Ayoade et al., 2014	*Pseudomonas fluorescens*	Subramanian et al., 2009; Prem Anand et al., 2010; Sakthivel et al., 2012; Liang et al., 2014
Citrobacter freundii	Prem Anand et al., 2010; Ayoade et al., 2014	*Rhodococcus* spp.	Liang et al., 2014
Corynebacterium spp.	Kalpana et al., 1994; Feng et al., 2011	*Serratia liquefaciens*	Prem Anand et al., 2010
Enterobacter spp.	Prem Anand et al., 2010; Feng et al., 2011; Pradhap & Selvisabhanayakam, 2011; Liang et al., 2014	*Serratia marcescens*	Ayoade et al., 2014; Zhang et al., 2020
Enterobacter cloacae	Ayoade et al., 2014	*Staphylococcus* spp.	Feng et al., 2011
Enterobacteriaceae	Kalpana et al., 1994	*Staphylococcus albus*	Priyadharshini et al., 2008
Enterococcus spp.	Li et al., 2015	*Staphylococcus aureus*	Priyadharshini et al., 2008; Sakthivel et al., 2012
Enterococcus casseliflavus	Liang et al., 2018	*Staphylococcus epidermidis*	Ayoade et al., 2014
Enterococcus faecalis	Liang et al., 2018	*Staphylococcus gallinarum*	Liang et al., 2018
Enterococcus mundtii	Fei et al., 2006; Cappellozza et al., 2011; Liang et al., 2018	*Stenotrophomonas* spp.	Feng et al., 2011
Escherichia coli	Prem Anand et al., 2010; Sakthivel et al., 2012	*Streptococcus pneumoniae*	Sakthivel et al., 2012
Erwinia spp.	Prem Anand et al., 2010; Liang et al., 2015	*Streptococcus sanguinis*	Liang et al., 2018
Klebsiella spp.	Feng et al., 2011	*Streptomyces* spp.	Subramanian et al., 2009

Rattana et al. (2017) carried out a study to test the toxicity of powder from silkworm larvae. Acute and subchronic toxicity studies on Sprague-Dawley rats with, respectively, a single dose up to 2,000 mg/Kg and a repeated dose up to 2,000 mg/Kg twice a day for 6 weeks, resulted in no toxicity (Rattana et al., 2017).

In the third study investigating toxicity, researchers carried out a sub-acute toxicity test with a dose of dried pupae of 3000 mg/Kg/day for 28 days and reported that no toxicity was detected (Jiang et al., 2020).

3.2.3. Results of individual studies on chemical contaminants

3.2.3.1. Contaminants in marketed edible B. Mori. Two studies investigated contamination of marketed edible insects (Aydoğan, 2021; Chomchai & Chomchai, 2018). One study described an outbreak of histamine poisoning due to consumption of street food in Thailand, with 28 of 227 students showing at least one symptom. Following a survey among exposed students, a relative risk ratio (RRR) of 16 (8.8–29.3) was associated with the consumption of silkworm pupae and a RRR of 18.7 (9.6–36.4) with the consumption of grasshoppers. The analysis of silkworm samples revealed total histamine contents of 76.6 mg/Kg in silkworm and of 97.3 mg/Kg in grasshopper. According to the authors, analyses from a previous (2007) outbreak resulted in a silkworm pupae histamine content up to 875 mg/Kg. Analyses for residues of organophosphorus, carbamate, and pyrethroid insecticides were negative. It should be noted that the insects, along with other foodstuffs sold by the street vendors, had been marinated in fish sauce during preparation, and although the other foodstuffs were associated with low histamine contents, the role of fish sauce cannot be completely neglected (Chomchai & Chomchai, 2018).

A second study investigating marketed edible insects used energy dispersive X-ray fluorescence and reported the results of a survey on the heavy metal contents of edible insect products bought online. The following concentrations of heavy metals were determined in one sample of BM pupae: lead (Pb) 0.4 mg/Kg, arsenic (As) 0.3 mg/Kg, bromine (Br) 0.05 mg/Kg, chromium (Cr) 0.3 mg/Kg, vanadium (V) 0.3 mg/Kg, titanium (Ti) 0.4 mg/Kg (Aydoğan, 2021). All the remaining studies had a similar study design aimed at evaluating the bioaccumulation of heavy metals along the silkworm production chain, mainly for environmental purposes.

3.2.3.2. Heavy metal bioaccumulation in B. Mori

3.2.3.2.1. Arsenic (As). Two studies investigated the bioaccumulation of As in silkworm (Feng et al., 2019; Wan et al., 2017) (Table 9). In both cases soil contamination was natural, although, in the

Table 4
List of bacteria genera and/or species detected in B. mori-based food through non-metagenomic analysis.

Bacteria detected in insect-based food	N detecting/N investigating*	Type of insect-based food		References
		Positive	Negative	
Bacilli	1/1	Dried; deep frozen; canned	Tsukudani	Grabowski & Klein 2017a
Bacillus cereus	2/3	Boiled; dried; salted; cooked and dehydrated	Dried; tsukudani; deep frozen; canned	Grabowski & Klein 2017a; Osimani et al., 2018a; Fasolato et al., 2018°
Bacillus lehensis	1/1	Boiled; dried; salted		Osimani et al., 2018a
Clostridium spp.	1/2	Frozen	Cooked and dehydrated	Fasolato et al., 2018; Kurdi et al., 2021
Coxiella burnetii	0/1		Boiled; dried; salted	Osimani et al., 2018a
Corynebacterium spp.	1/1	Boiled; dried; salted		Osimani et al., 2018a
Enterobacteriaceae	2/3	Dried; deep frozen; canned; frozen	Tsukudani; cooked and dehydrated	Grabowski & Klein 2017a; Fasolato et al., 2018; Kurdi et al., 2021
Escherichia coli	0/1		Dried; tsukudani; deep frozen; canned	Grabowski & Klein 2017a
Geobacillus spp.	1/1	Boiled; dried; salted		Osimani et al., 2018a
Lactic Acid Bacteria	1/2	Cooked and dehydrated	Frozen	Fasolato et al., 2018; Kurdi et al., 2021
Listeria spp.	0/1		Cooked and dehydrated	Fasolato et al., 2018
Listeria monocytogenes	0/1		Dried; tsukudani; deep frozen; canned	Grabowski & Klein 2017a
Paenibacillus apiarius	1/1	Boiled; dried; salted		Osimani et al., 2018a
Pseudomonas aeruginosa	0/1		Boiled; dried; salted	Osimani et al., 2018a
Salmonella spp.	0/2		Dried; tsukudani; deep frozen; canned; cooked and dehydrated	Grabowski & Klein 2017a; Fasolato et al., 2018
Staphylococcus spp.	1/1	Cooked and dehydrated		Fasolato et al., 2018
Staphylococcus aureus	0/2		Dried; tsukudani; deep frozen; canned; cooked and dehydrated	Grabowski & Klein 2017a; Fasolato et al., 2018
Shiga toxin-producing *E. coli* (STEC)	0/1		Boiled; dried; salted	Osimani et al., 2018a

*No. of studies detecting / no. of studies investigating bacteria type.

case of Wan and colleagues, the contamination level was increased by a concurrent flooding event that caused heavy metal release from local mines (Wan et al., 2017). Feng and colleagues reported a leaf As concentration of 0.33 mg/Kg, and after 10 days of feeding, a silkworm body As concentration of 1.83 mg/Kg in pupae and 0.69 mg/Kg in feces (Feng et al., 2019). Wan and colleagues reported As concentrations in leaf of 0.12 mg/Kg (young leaf) and 0.34 mg/Kg (old leaf). In silkworm, the highest concentration was 0.71 mg/Kg, whereas in control insects it was 0.52 mg/Kg. Differences among concentration in different insect species were not statistically significant (Wan et al., 2017). In both studies, a bioaccumulation potential for As in pupae was observed.

3.2.3.2.2. Cobalt (Co). The potential bioaccumulation in silkworm of cobalt (Co) from contaminated soil was investigated in a single study (Table 9) where soil with an initial Co concentration of 8.54 mg/Kg was irrigated with water containing different Co concentrations ranging from 25 to 400 mg/L (Ashfaq et al., 2009b). Silkworms were exposed during their whole life cycle. At the highest soil concentration (273.5 mg/Kg after 75 days of irrigation), the accumulation in *Morus alba* (mulberry) leaves was up to 42.85 mg/Kg; in BM larva up to 31.2 mg/Kg; in feces up to 19.76 mg/Kg. Body weight and length were reduced in the exposed insects. Despite not being a great Co bioaccumulator, mulberry could be a Co source for silkworms in whose body the metal can be recovered (Ashfaq et al., 2009b).

3.2.3.2.3. Chromium (Cr). One study was carried out with Cr III (Table 9), the content of which in irrigation water ranged from 25 to 200 mg/L, and which resulted in the accumulation of: up to 100 mg/Kg in soil; up to 70–80 mg/Kg in *M. alba* leaves; up to 61 mg/Kg in larvae exposed for the whole life cycle, and; up to 59 mg/Kg in feces. Negative effects were observed on body length, weight, and death rate. Despite a noteworthy excretion rate via feces, the Cr content in the silkworm body was remarkable (Ashfaq et al., 2012).

3.2.3.2.4. Copper (Cu). Soil amended with copper (Cu) (5 to 320 mg/Kg) was used for mulberry cultivation and resulting leaves were fed to BM from the 3rd larval instar onward. A mobility index was calculated by dividing the concentration in the receiver by the concentration in the source. The highest mobility indices were observed in the transfer from soil to roots (up to 2.13 with a soil concentration of 5 mg/Kg) and from leaves to larvae (up to 3.12 with a soil concentration of 80 mg/Kg) (Prince et al., 2001). Study characteristics are reported in Table 9.

3.2.3.2.5. Zinc (Zn). Zinc (Zn) bioaccumulation was investigated by two studies (Table 9). Ashfaq and colleagues used soil that was contaminated with irrigation water (Zn at 25 to 400 mg/L) and found accumulation of up to 386 mg/Kg in soil, up to 142 mg/Kg in *M. alba* leaves, up to 91 mg/Kg in BM larvae fed with leaves from contaminated soil, and up to 42 mg/Kg in feces. Effects were observed on the body length, weight, and death rate (Ashfaq et al., 2010). The second study dealing with Zn was carried out by feeding BM with leaves from soil contaminated after a catastrophic flooding event hitting local mines. The amount of Zn in young leaf was up to 65 mg/Kg whereas in old leaf it was up to 94 mg/Kg. Up to 214 mg/Kg was found in exposed larvae, whereas up to 81 mg/Kg occurred in control larvae, fed with leaves containing 39 mg/Kg of Zn (Wan et al., 2017). Therefore, Zn bioaccumulation by BM larvae was observed.

Table 5
Bacteria counts reported for B. mori unprocessed insects and insect-based food. Bacteria counts of insect-based foods are reported only for foods containing exclusively B. mori.

	UNPROCESSED INSECTS		
Type of target analysis	**Count**	**Note**	**Reference**
Total bacteria count	$10^4 \leq X \leq 2x10^7$ CFU/g*	Body surface	Ayoade et al., 2014
	$10^4 \leq X \leq 10.03x10^8$ CFU/g*	Gut	
	$10^4 \leq X \leq 0.79x10^8$ CFU/g*	Whole body	
	6.3×10^7 CFU/ml	Fluoride resistant strain – fluoride treated	Li et al., 2016
	7.1×10^7 CFU/ml	Fluoride resistant strain – water treated	
	4.8×10^7 CFU/ml	Fluoride susceptible strain – fluoride treated	
	7.5×10^7 CFU/ml	Fluoride susceptible strain – water treated	
	6.080×10^{11} CFU/ml		Prem Anand et al., 2010
	7.1×10^7 CFU/ml		Liang et al., 2014
	3.2×10^7 CFU/ml	Lettuce diet from 4th instar	
	3.33×10^1 CFU/g	Germ free artificial diet	Cappellozza et al., 2011
	1.60×10^7 CFU/intestine		
	5.7×10^4 CFU/g	1st instar	Kalpana et al., 1994
	4.2×10^5 CFU/g	2nd instar	
	4.6×10^5 CFU/g	3rd instar	
	1.8×10^6 CFU/g	4th instar	
	1.3×10^7 CFU/g	5th instar	
Anaerobic	2.7×10^6 CFU/ml		Prem Anand et al., 2010
	INSECT-BASED FOOD		
Type of target analysis	**Count**	**Note**	**Reference**
Total bacteria count	10^6 CFU/g	Canned silkworms	Grabowski & Klein, 2017a
	$10^5 \leq X \leq 10^6$ CFU/g	Deep frozen silkworms	
	3.0×10^2 CFU/g		Milanović et al., 2016
	10^6 CFU/g approx.**	Cooked and dehydrated; not declared	Fasolato et al., 2018
	1.34×10^8 CFU/g		Kurdi et al., 2021
Aerobic spores	10^5 CFU/g approx.**	Cooked and dehydrated; not declared	Fasolato et al., 2018
Yeasts and molds	7.08×10^5 CFU/g		Kurdi et al., 2021
	<10 CFU/g	Canned silkworms	Grabowski & Klein, 2017a
	10^2 CFU/g	Deep frozen silkworms	
Enterobacteriaceae	10^7 CFU/g	Canned silkworms	Grabowski & Klein, 2017a
	$10^2 \leq X \leq 10^3$ CFU/g	Deep frozen silkworms	
	< 10 CFU/g	Cooked and dehydrated; not declared	Fasolato et al., 2018
	5.13×10^4 CFU/g		Kurdi et al., 2021
Clostridium	2.14×10^3 CFU/g		Kurdi et al., 2021
	<10 CFU/g	Cooked and dehydrated; not declared	Fasolato et al., 2018
Bacillus cereus	$10^2 \leq X \leq 10^3$ CFU/g	Cooked and dehydrated; not declared	Fasolato et al., 2018
Bacilli	10^7 CFU/g approx.	Canned silkworms	Grabowski & Klein, 2017a
	10^3 CFU/g	Deep frozen silkworms	

*range of counts for different B. mori strains.
**see Figure 2 (boxplot) in the text ofFasolato et al., 2018.

3.2.3.2.6. Cadmium (Cd). Cadmium (Cd) bioaccumulation was investigated in six studies (Ahmad et al., 2020; Feng et al., 2019; Y. Jiang et al., 2020; Prince et al., 2001; Si et al., 2021; Suzuki et al., 1984) (Table 10). Five studies reported data useful for evaluating bioaccumulation, while the sixth study observed increases in Cd concentrations in the alimentary canal (1,100 μg/g dry weight) and in Malpighian tube (470 μg/g dry weight) (Suzuki et al., 1984). The two studies with natural soil contamination (Feng et al., 2019; Jiang et al., 2020) observed a transfer factor < 1 from leaf to silkworm and > 1 from silkworm to feces. The three studies investigating bioaccumulation following artificial contamination of soil with irrigation water showed conflicting results. Ahmad and colleagues found no bioaccumulation (Ahmad et al., 2020); Prince and colleagues found a transfer factor > 1 for both, leaf to larva and larva to feces (Prince et al., 2001); Si and colleagues found a transfer factor < 1 only from larva to feces (Si et al., 2021).

3.2.3.2.7. Lead (Pb). Pb bioaccumulation was investigated in six studies (Ashfaq, Khan, et al., 2009; Feng et al., 2019; Jiang et al., 2020; Si et al., 2021; Wan et al., 2017; Zhou et al., 2015) (Table 11). Three studies investigated Pb transfer along the chain starting from naturally contaminated soil (Feng et al., 2019; Jiang et al., 2020; Wan et al., 2017). No bioaccumulation was seen from leaf to silkworm, whereas the transfer index from larva to feces was > 1. This result was confirmed also by two studies dealing with artificial soil contamination (Si et al., 2021; Zhou et al., 2015). Only one study identified a potential for Pb bioaccumulation from leaf to silkworm (Ashfaq et al., 2009a). The collected evidence suggests that Pb is efficiently eliminated by BM through feces, but its presence in the larva should not be neglected.

3.2.4. Synthesis of results

The three studies dealing with toxicity did not highlight any concern in investigated animals following the ingestion of BM powder. These preliminary results suggest that toxicity due to BM consumption is unlikely.

Data on prevalence and real scenarios about concentrations of heavy metals in BM are scarce. However, in most cases metals were efficiently eliminated through silkworm feces, with the exception of As (Feng et al., 2019; Wan et al., 2017), Cu (Prince et al., 2001) and Zn (Wan et al., 2017), which have bioaccumulation potential and so raise some concerns and need further investigation. It should be noted that mulberry (and silkworm) contamination was due to the cultivation on artificially- or naturally-contaminated soils and that even in that critical situation, for most heavy metals, bioaccumulation was not detected. This is because mulberry is known to have a high tolerance toward heavy metals in soil, which is why the tree is often cultivated for soil bioremediation purposes.

Table 6
General characteristics of studies dealing with toxicity. NR: Not Reported.

Reference	Insect Species (strain)	Diet/Feed	Life cycle stage	Effect	In vitro Toxicity target	In vivo Toxicity target	Methods
Heo et al., 2013	*B. mori* (strain: YeonNokJam)	Mulberry leaves	Larva 5th instar	Acute and sub-acute toxicity	*Salmonella* (Ames test), cell culture	Rats	Direct observation for any symptomatology; urine and blood parameter analyses; necroscopic analysis
Jiang et al., 2020	silkworm strain P50	Mulberry leaves	Pupa	Acute and sub-chronic toxicity	–	Rats	Toxicity study in rats
Rattana et al., 2017	*B. mori*	NR	Larva 5th instar	Acute and sub-acute toxicity	–	Rats	Microtiter plate reading for absorbance

3.3. Allergenic hazards associated with B. Mori consumption

3.3.1. Study characteristics

Overall, 16 papers dealt with allergological hazards related to BM. Studies were then categorized based on the following criteria: reports of people with allergic symptomatology following the ingestion of BM (3 studies); characterization of potential allergenic determinants in BM (13 studies); studies reporting the effect of thermal processing on the allergenicity of BM proteins (2 studies).

3.3.2. Results of individual studies on allergies following the ingestion of B. Mori

Three studies referred to specific cases of people with allergic symptomatology following the ingestion of BM (Table 12). In two studies, patients were from the United States of America (USA) (Dietrich et al., 2008; Gautreau et al., 2017), while the remaining study was from China (Ji et al., 2009). The growth stage of the consumed BM was reported by two studies as "pupa". Although in one study the actual food matrix was not mentioned (Gautreau et al., 2017), in another one, the patient showed allergic symptomatology after having eaten canned silkworm pupae (Dietrich et al., 2008), while in the third, the cause was ingestion of oil-fried silkworm pupae (Ji et al., 2009). The latter study also reported 13 cases of Chinese citizens who had severe anaphylactic reactions following the ingestion of silkworm pupae. However, the exact way in which BM pupae were cooked was not mentioned. Interestingly, in just one study did the authors check for a possible correlation between the allergic reaction and BM, via skin prick test (SPT) (Dietrich et al., 2008). Symptomatology included a wide plethora of symptoms, with flushing being the most common.

3.3.3. Results of individual studies on potential allergenic determinants of B. Mori

Thirteen studies dealt with the identification and characterization of potential allergenic determinants (proteins) in BM (Table 13). Since sensitization to a specific food allergen does not occur only through oral exposure, but also via skin contact or inhalation (de Gier & Verhoeckx, 2018), we included studies dealing with all these possible routes of exposure. Ten studies were from Asian countries, while the remaining three were from Europe, and South or North America. Three studies focused their investigation on BM moths (Araujo et al., 2020; Komase et al., 1997; Venkatappa et al., 2005), one on larvae (Liu et al., 2009), whilst the remaining 9 were on pupae (Barre et al., 2021; He et al., 2021; Jeong et al., 2016, 2017; Komase et al., 1997; Ling et al., 2019; Wang et al., 2016; Yigit et al., 2021; Zhao et al., 2015; Zuo et al., 2015). In three, even though the authors managed to identify IgE-binding proteins of BM by assigning them certain molecular weights, no name regarding any possible relative molecule was given (He et al., 2021; Komase et al., 1997; Venkatappa et al., 2005). As for the other 10, at least one potential allergenic molecule was both weighted and named. With regard to the named, potentially allergenic proteins, speculation or data about cross-reactivity with similar molecules in other taxa were also reported by some studies. One study, in particular, made a thorough assessment in this sense (Barre et al., 2021), since the authors managed to identify 161 proteins in an extract of BM pupae, and of these, 37 were considered as IgE-binding allergens that cross-reacted with proteins from other edible insect species and were also typical of other taxa (crustaceans, acari, fungi, and molluscs) listed by the authors. As for other studies, Araujo et al. (2020) speculated about the similarity between BM's vitellogenin and that of *Galleria mellonella*; Jeong et al. (2016) found amino acid similarities among a 27 kDa glycoprotein in BM and those from other Lepidoptera species, such as *G. mellonella*; Jeong et al. (2017) observed the potential cross-reactivity between a BM tropomyosin and crustacean tropomyosins (shrimps and crabs); Liu et al. (2009) reported how an arginine kinase from BM cross-reacted with its analog in the cockroach *Periplaneta americana*; Zhao et al. (2015) observed a resemblance of the BM chitinase to a protein from the mite *Dermatophagoides farinae* (24.8 % amino acid identity and 57.4 % similarity), as well as a similarity between BM paramyosin and a protein from the mite *Dermatophagoides pteronyssinus* (62.8 % amino acid identity and 90.0 % similarity). Finally, Zuo et al. (2015) did not find Bom m 9 (a member of the 30 k family of silkworm proteins and which is accumulated in the insect hemolymph) was cross reactive with moth or cockroach in immunoblot inhibition assays; however, the amino acid sequence of this protein had a high similarity with the microvitellogenin of the moth, *Manduca sexta.*

3.3.4. Results of individual studies on thermal effects on the allergenicity of B. Mori

Two studies were included in this category and results are reported in Table 14**.** One study assessed the effect of boiling on silkworm pupae protein extract (SPPE), and allergenicity of protein components was evaluated through IgE immunoblotting with sera of BM sensitive patients (Jeong et al., 2016). The SPPE was boiled for 5 min (temperature was not reported), and the authors observed an increase in the IgE reactivity to a 27 kDa hemolymph glycoprotein, with respect to the untreated SPPE. In the second study, He et al. (He et al., 2021) tested the effects of heating SPPE for different temperatures and times. Allergenicity was then investigated through SDS-page, Western Blotting, and ELISA. Their results showed how IgE-binding proteins between 25 and 33 kDa were thermostable at temperatures below 100 °C and after 30 min of heat treatment.

3.3.5. Synthesis of results

Three studies reported about people with allergic reactions following the ingestion of BM, even though in two there was no diagnostic verification of the etiological agent responsible for the allergic reactions. Studies regarding the description of potential allergenic determinants in BM were also assessed. In fact, the BM proteins arginine kinase and tropomyosin cross-react, respectively, with the same proteins from the cockroach *P. americana* and from shellfish (Jeong et al., 2017; Liu et al., 2009). Furthermore, researchers (Barre et al., 2021) point out how BM has a high number of allergenic proteins that are also shared with other insect species, and cross-reactivity with other organisms such as crustaceans, acari, molluscs, and fungi seems to be widespread. This indicates that, for the greatest part, these proteins are pan-allergens.

As for thermal treatments, two studies indicate that heat does not impair the allergenicity of BM protein determinants; indeed, it seems to

Table 7
General characteristics of studies dealing with chemical contamination. NR: Not Reported. AAS: Atomic absorption spectroscopy. EDXRF: Energy dispersive X-ray fluorescence. ICP-MS: Inductively Coupled Plasma-Mass Spectrometry. AFS: Atomic fluorescence spectrometry. AES: Atomic emission spectrometry. HPLC: High Performance Liquid Chromatography.

Reference	Insect Species	Sample origin	Diet/Feed	Life cycle stage	Origin of contamination	Hazard description	Methods
Ahmad et al., 2020	*B. mori*	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Cd bioaccumulation	AAS
Ashfaq et al., 2009a	*B. mori*	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Pb bioaccumulation	AAS
Ashfaq et al., 2009b	*B. mori*	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Co bioaccumulation	AAS
Ashfaq et al., 2010	*B. mori*	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Zn bioaccumulation	AAS
Ashfaq et al., 2012	*B. mori*	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Cr bioaccumulation	AAS
Aydogan 2021	*B. mori* (12 in total)	Bought online	NR	Pupa	Unknown	Presence of metals: Pb, As, Br, Cr, V, Ti	EDXRF
Chomchai and Chomchai, 2018	*B. mori*	Street food	NR	Pupa	Processing	Histamine - Acute poisoning	Interviews and chemical analysis, fluorimetry
Feng et al., 2019	*B. mori*	Reared under experimental conditions	Mulberry leaves	Pupa	Soil	As Bioaccumulation	ICP-MS
Feng et al., 2019	*B. mori*	Reared under experimental conditions	Mulberry leaves	Pupa	Soil	Pb Bioaccumulation	ICP-MS
Feng et al., 2019	*B. mori*	Reared under experimental conditions	Mulberry leaves	Pupa	Soil	Cd Bioaccumulation	ICP-MS
Jiang et al., 2020	Silkworm	Reared under experimental conditions	Mulberry leaves	Larva and Moth	Soil	Pb Bioaccumulation	AAS
Jiang et al., 2020	Silkworm	Reared under experimental conditions	Mulberry leaves	Larva and Moth	Soil	Cd Bioaccumulation	AAS
Wan et al., 2017	NR	Reared under experimental conditions	Mulberry leaves	Pupa	Soil	As bioaccumulation	AFS, ICP-MS
Wan et al., 2017	NR	Reared under experimental conditions	Mulberry leaves	Pupa	Soil	Pb bioaccumulation	AFS, ICP-MS
Wan et al., 2017	NR	Reared under experimental conditions	Mulberry leaves	Pupa	Soil	Zn bioaccumulation	AFS, ICP-MS
Prince et al., 2001	*B. mori* (Bivoltine hybrid race)	Reared under experimental conditions	NR	Larva	Soil	Cd bioaccumulation	AAS
Si et al., 2021	*B. mori* Jing-song × Haoyue	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Pb bioaccumulation	–
Si et al., 2021	*B. mori* Jing-song × Haoyue	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Cd bioaccumulation	–
Suzuki et al., 1984	*B. mori*	Reared under experimental conditions	Artificial diet (sterile) mulberry feed powder	Larva	Soil	Cd accumulation	ICP-AES; HPLC-AAS method;
Zhou et al., 2015	Hybrid silkworm	Reared under experimental conditions	Mulberry leaves	Larva	Soil	Pb bioaccumulation	ICP-AES

enhance their allergenic potential, and thus, these proteins pose an even greater risk when BM is cooked.

4. Discussion

Microbiological, chemical, and allergenic hazards identified and discussed in the present review do not raise safety concerns that would impair the potential use of BM as food. Identified hazards can be managed to guarantee the safety of BM derived products as is commonly done for other foods usually consumed by the population. However, our systematic review reveals a lack of evidence, in particular in the specific context of BM farmed for food production.

Concerning microbiological hazards, using as a reference the microbiological criteria of other insect species authorized as Novel food in Europe (European Commission (EU), 2017), we can state that, as reported in Table 5, some samples of insect based-food analyzed in included studies exceeded the count limits of the following microbial target: total aerobic colony count (canned, deep frozen, cooked and

Table 8
Results of studies dealing with toxicity. NR: Not reported. PSP: Protein of silkworm pupae.

Reference	Matrix tested	Study	Organism Tested	N	Doses	Acute Toxicity doses tested	Duration	Observation time	Endpoints	Results
Rattana et al., 2017	Powder of 5th instar silkworm larvae	Acute-toxicity test	Male albino Wistar rats	6x10	Single dose	Three doses of 1000; 1500; 2000 mg/Kg bw	NR	15 days	Behavioral changes and general toxicity	No toxicity in rats tested with acute and sub-acute silkworm powder doses
Rattana et al., 2017	Powder of 5th instar silkworm larvae	Sub acute-toxicity test	Male albino Wistar rats	6x10	Repeated dose	2000 mg/Kg bw; 2 doses/day	6 weeks	Every week	–	No toxicity
Heo et al., 2013	Lyophilized powder	Acute toxicity	Sprague-Dawley rats (SPF)	4x10	Single dose	Doses of 0; 1250; 2500; 5000 mg/Kg	NA	15 days	Mortality, body weight, gross lesions	No toxicity in rats tested with acute doses. Lethal dose > 5000 mg/Kg
Heo et al., 2013	Lyophilized powder	Subchronic toxicity	Sprague-Dawley rats (SPF)	4x20	Repeated dose	0; 500; 1000; 2000 mg/Kg/day	90 days	daily, weekly, end of experiments	Mortality, clinical signs, body weights, urine analysis, gross lesions, serum, blood	No toxicity in rats tested with sub-acute powder doses. NOAEL set to be 2000 mg/Kg/day
Heo et al., 2013	Lyophilized powder	Reverse mutation test	*Salmonella* Typhimurium TA100, TA1535, TA98, TA1537 (22) and a tryptophan auxotroph strain of *Escherichia coli* WP2 uvrA	NR	NR	5000, 1500, 500, 150, 50 and 10 mg/plate	NR	NR	Mutations	No toxicity
Heo et al., 2013	Lyophilized powder	Chromosomal aberration assay	Chinese hamster lung cells (CHL/IU)	NR	NR	0, 150, 275, 300, 550, 600, 700, 900, 1100 μg/ml	NR	0–24 h	Aberrant metaphases	No toxicity
Heo et al., 2013	Lyophilized powder	Bone marrow micronucleus assay	ICR mice (SPF)	6	NR	1250, 2500 and 5000 mg/Kg	NR	2 days	Mortality and abnormality	No toxicity
Jiang et al., 2020	Dried Pupae	Sub acute-toxicity test	Sprague-Dawley rats (SPF)	20x4	Repeated dose	3000 mg/day (PSP 50 %)	28 days	–	Body weight, feed consumption, hematology, Serum biochem parameter, organ weight, histopathologic examination	No toxicity

Bw: body weight.

dehydrated silkworms); *Enterobacteriaceae* (canned, deep frozen silkworms); presumptive *B. cereus* (cooked and dehydrated silkworms); sulfite reducing anaerobes (see *Clostridium* count in one sample); yeasts and molds (deep frozen silkworms).

Salmonella spp. and *L. monocytogenes*, two of the pathogens most implicated in cases of food outbreaks in Europe (Schirone & Visciano, 2021), were never detected in the included studies. The absence of *Salmonella* spp. and *L. monocytogenes* is in accordance with the fact that mass-reared insects are not likely to contain substantial numbers of zoonotic pathogens in comparison to warm-blooded animals. If contamination with pathogens were to occur, this would be mainly related to an infection from the production environment, involving a low number of bacteria types (NVWA, 2014). In addition, although zoonotic pathogens can be detected in the substrates used to grow insects, active replication of the pathogens in the intestinal tract of insects does not seem to occur, but these animals can play a role as vehicles (EFSA, 2015; Mancini et al., 2019).

High contamination levels of aerobic spores (10^5 CFU g), *B. cereus* (10^2 –10^3 CFU/g), and *Enterobacteriaceae* (10^7 CFU/g) were observed for insect-based food investigated in the included studies. The presence of aerobic spores is also supported by the detection of many species belonging to *Bacillales* order in the included studies (see Table 3 and 4). Similar counts of aerobic spores and *B. cereus* were also reported for the European most common edible insect species, as for example, in processed *Acheta domesticus* (Osimani et al., 2017; Osimani et al., 2018b) and *Tenebrio molitor* (Stoops et al., 2016), and raw *A. domesticus*

Table 9
Results of studies dealing with accumulation of heavy metals, results in mg/Kg.

Reference	Soil contamination	Exposure	Soil	Content mg/Kg Dry weight			Bioaccumulation			Contaminant
				Leaves (dry weight)	Silkworm (dry weight)	Feces (dry weight)	Soil-Leaf	Leaf-Silkworm	Silkworm-feces	
Feng et al., 2019	Natural	10 days (3 times a day)	31–62	0.14–0.33	Pupa 1.83 (1.52–1.83)	0.40–0.69	No	Yes	No	As
Wan et al., 2017	Natural (flood)	45 days	14.3–52.8/ 3.5–16,8 (control)	0.12–0.34/ 0.17–0.30 (control)	Pupa 0.71/ 0.52 (control)	0.67/0.66 (control)	No	Yes	No	As
Ashfaq et al., 2009b	Artificial (Soil with initial Co 8.45 mg/Kg, irrigated with water with Co from 25 to 400 mg/L)	Whole life cycle	59.5–273.5	15.45–42.85	Larvae (5th instar) 11.7–31.2	NR-19.76	No	No	No	Co
Ashfaq et al., 2012	Artificial (Soil with initial Cr 15,56 mg/Kg, irrigated with water with Cr from 25 to 200 mg/l)	Whole life cycle	99.89	70–80	Larvae (5th instar) 61.32	58.97	No	No	No	Cr (III)
Prince et al., 2001	Artificial (Leaves form mulberry grown in soil amended with 5, 10, 20, 40, 80, 160, 320 mg/Kg)	From 3rd instar	3.10–52	4.16–14.60	Larva 6.52–32.20	5.26–20	No	Yes	No	Cu
Ashfaq et al., 2010	Artificial (Soil with initial Zn 15.56 mg/ Kg, irrigated with water with Zn from 25 to 400 mg/L)	Whole life cycle	386.51	142.85	Larvae (5th instar) 91,37	42.13	No	No	No	Zn
Wan et al., 2017	Natural (flood)	45 days	99–1,194/ 27.3–328.1 (control)	65,87–94/ 38.55–38.72 (control)	Pupa 213.9–80.6 (control)	126.4–38 (control)	No	Yes	No	Zn

Table 10
Results of studies dealing with accumulation of cadmium, results in mg/Kg.

Reference	Soil contamination	Exposure	Soil	Leaves (dry weight)	Silkworm (dry weight)	Feces (dry weight)	Soil-Leaf	Leaf-Silkworm	Silkworm-feces	Results
Ahmad et al., 2020	Artificial (Soil with initial Cd 0.75 mg/Kg, irrigated with water with Cd from 25 to 200 mg/L)	Whole life cycle (22–28 days)	45.32–99.89	16,34–95,67	Larva (5th instar) 3.1–61.32	10.18–58.97	No	No	No	Level increasing at growing pH.
Feng et al., 2019	Natural	10 days	NR	0,7–0,21	Pupa 0.02–0.03	0.12–0.20	NR	No	Yes	Excrements 86–92; pupa 5–8 %; cocoon 0–8 %
Jiang et al., 2020	Natural	8 days	3.22 (±0.92)	0.14–0.23	Larva 0.01–0.43; Pupa 0.02–0.05	0.11–0.19	No	No	Yes	–
Prince et al., 2001	Artificial (soil polluted with 5 to 320 ppm Cd)	From 3rd instar	0.750–11.780	4.66–9	Larva 2.46–20.4	7.20–3.23	No	Yes	No*	* overall (across life stages) Cd mobility index: leaf to larva 1.60, larva to feces 1.16. No data for contamination 320 mg/Kg
Si et al., 2021	Artificial contamination through irrigation water with 0 to 75 mg/L of Pb.	larva 1st to 5th instar	NR	NR	NR	NR	No	No	Yes	Decrease in length and weight. Cd (3.73 %– 13.74 %) was transferred from the soil to the mulberry, of which 21.77 %– 26.96 % into the mulberry leaves. Cd in silkworm body 18.00 % –45.52 %. 54.48 %–82.00 % excreted in feces.

Table 11
Results of studies dealing with accumulation of lead, results in mg/Kg or %.

Reference	Soil contamination	Soil	Leaves (dry weight)	Silkworm (dry weight)	Feces (dry weight)	Soil-Leaf	Leaf-Silkworm	Silkworm-feces	Exposure	Results
Ashfaq et al., 2009a	Artificial contamination through irrigation water with 25 to 400 mg/L of Pb.	326.5	42.78	60–65	20–25	No	Yes	No	Whole life cycle	Accumulation in soil is pH dependent; maximum at 5. Pb toxicity in BM observed
Feng et al., 2019	Natural	NR	1.76–2.91	Pupa 0.10–0.34	2.60–8.47	NR	No	Yes	10 days	–
Jiang et al., 2020	Natural	181.23 (40)	1.45–4.48	Larva 5th instar 0.09–0.54: Pupa 0.23–0.49	3.14–7.09	No	No	Yes	8 days	–
Si et al., 2020	Artificial contamination through irrigation water with 0 to 1000 mg/L of Pb.	NR	NR	NR	NR	No	–	Yes	larva 1st to 5th instar	Decrease in length and weight (not at high concentration). Under single Pb stress, 12.87 % −18.01 % of the Pb in the soil was transferred to mulberry, of which roots stored 65.54 %−75.24 % Pb, stem contained 6.53 %−13.00 % and 18.23 %−21.51 % was stored in mulberry leaves. As the silkworm fed on mulberry leaves, 8.67 %− 11.19 % of the Pb into the silkworm, and most of the Pb (over 80 %) were eliminated with the silkworm frass
Wan et al., 2017	Natural (flooding event)	81–733/ 6.1–48.7 (control)	4.32–11.87/ 3.32–6.49 (control)	0.48/0.45 (control)	12.3/9.9 (control)	No	No	Yes	45 days	0.48 (flood inundated) vs 0.45 (control) mg/Kg. No difference
Zhou et al., 2015	Artificial. Soil incubated with 200 to 800 mg/Kg Pb	204/758	41–79/60.26	Larva 4.08–11.16; moth 2.95–6.23	187.96–279.80	No	No	Yes	5 days (5th instar)	In larva up to 11.16 mg/Kg, in feces up to 280, in silk moth up to 6.23.

Table 12
Reported cases of foodborne allergic episodes due to B. mori.

Reference	Country	Patients	Life cycle	Food matrix	Allergy confirmation/ Method	Reported Symptomatology
Dietrich et al., 2008	USA	1	Pupa	canned silkworm pupae	Yes/SPT*	Flushing; facial angioedema; ocular pruritus; erythema; nasal congestion; throat tightness
Gautreau et al., 2017	USA	1	Pupa	not mentioned	No	urticaria; flushed and dry skin; high heart rate
		1				flushing; facial edema
Ji et al., 2009	China	1	Pupae	oil-fried silkworm pupae	No	pruritus; nausea, flush and swollen face; difficulty breathing
	China	1	Pupa	not mentioned	No	pruritus; urticaria; flushed appearance; hypotension; fainting; unconsciousness
	China	3	Pupa	not mentioned	No	urticaria; flushed appearance; headache; hypotension; abdominal pain; vomiting; dyspnea
	China	1	Pupa	not mentioned	No	pruritus; urticaria; fainting; hypotension; flushed appearance; nausea; unconsciousness
	China	8	Pupa	not mentioned	No	pruritus; urticaria; flushed appearance; angioedema; abdominal pain; vomiting; nausea; dyspnea

*SPT (Skin Prick Test).

(Klunder et al., 2012), *T. molitor* (Stoops et al., 2016), and *Locusta migratoria* (Stoops et al., 2016).

Concerning *Enterobacteriaceae*, high counts in other insect species were reported, as for example in processed insects (*L. migratoria*, *T. molitor, Alphitobius diaperinus*) at levels that exceeded 10^3 CFU/g (NVWA, 2014) and in raw *A. domesticus,* in which contamination levels of approx. 10^7 CFU/g were reported (Grabowski & Klein, 2017b; Vandeweyer et al., 2017; Vandeweyer et al., 2018).

Table 13
Studies dealing with the characterization of B. mori potentially allergenic determinants.

Reference	Country	Life cycle	Methods for protein characterization	Proteins characterized [Name/molecular weight (kDa)]	Cross Reactivity: possible correlations or protein similarities
Araujo et al., 2020	Brazil	moth	Western blotting of moth wing extract with sera from BM sensitive patients; MALDI-TOF for protein identification; search in NCBI and PDB databanks for protein matches	[no name/80; no name/66; no name/50; vitellogenin/45; no name/37; no name/30]	No [However, for vitellogenin there is a possible similarity with an IgE-reactive protein characterized in *G. mellonella* (moth). Almost all patients with sensitivity to mites presented reactivity with the *B. mori* 66 kDa protein]
Barre et al., 2021	France	pupa	SDS-page of pupae extract; protein analysis through nano-LC-MS/MS; Online database search for protein references	See Table 1 in the published article, for reference	Yes [37 proteins. See Table 2 in the published article, for reference][1]
He et al., 2021	China	pupa	Western blotting and ELISA of pupae extract with sera from BM sensitive patients	[no name for all/5 proteins between 20 and 95]	No
Jeong et al., 2016	South Korea	pupa	Immunoblotting of pupae extract with sera of BM sensitive patients; protein identification through LC-coupled ESI-MS/MS; recombinant *E. coli* for protein production; ELISA for specific IgE binding	[xanthine dehydrogenase/147; juvenile hormone epoxy hydrolase/52; cell differentiation protein/ 32; 27 kDa glycoprotein/24; putative cuticle protein/ 45]	Yes [52.6 %-56.6 % amino acid sequence identity of the 27 kDa glycoprotein with other Lepidopterans' glycoproteins, among which one from *G. mellonella* reported as an inhalant allergen]
Jeong et al., 2017	South Korea	pupa	Western blotting of pupae extract; recombinant *E. coli* for protein production; ELISA for IgE reactivity of recombinant protein; BLAST for protein alignment	[tropomyosin/36]	Yes [potential cross reactivity with shellfish tropomyosins (shrimp and crab)]
Komase et al., 1997	Japan	moth	Immunoblotting of moth extract with sera from BM sensitive patients	[no name for all/140; 130; 86; **82**; **79**; **76**; **72**; 55; 42; 38; 35.5; 32; 27; 22.5; 14 *	Yes [potential cross reactivity with the midge *Chironimus yoshimatsui]*
Ling et al., 2019	China	pupa	Pupae RNA was extracted and amplified through RT-qPCR; DNA fragments transformed in Rosetta cells for expression; Western blotting of pupae extract	[paramyosin/102; arginine kinase/40; glycoprotein/25; Jafrac1 (thiolperoxiredoxin)/22]	No
Liu et al., 2009	China	larva	Larvae arginine-kinase-RNA was extracted and *retro*-transcribed; recombinant *E. coli* for protein production; Protein alignment with Clustal X; Immunoblotting and ELISA of larvae extract with sera of BM sensitive patients	[no name/120; no name/110; no name/70; no name/50; arginine kinase/42	Yes [arginine kinase cross-reacts with that of *P. americana* (cockroach)]
Venkatappa et al., 2005	India	moth	Moth extract used for protein characterization through SDS-page	[no name for all/66; 45;31; 21; 14]	No
Wang et al., 2016	China	pupa	Immunoblotting of pupae extract with sera from BM sensitive patients; MALDI-TOF MS and MALDI-TOF MS/MS for protein characterization; Thiol peroxiredoxin (TP) DNA was transformed in BL21 for protein expression; ELISA with sera from BM sensitive patients for measuring TP specific-IgE	[uncharacterized protein/28.4; thiol peroxiredoxin/22; cuticolar protein precursor 21.5; heat shock protein/20.4; cuticolar protein precursor/18.5; chemosensory protein/14.5]	No
Yigit et al., 2021	USA	pupa/ cocoon	MS analysis of pupae extracts; BLAST and bioinformatic analysis for protein identification	[tropomyosin; arginine kinase; thiolredoxin; chitinase; paramyosin/no molecular weight]	No
Zhao et al., 2015	China	pupa	Western blotting of pupae extract with sera from BM sensitive patients; MALDI-TOF MS for protein analysis and identification	[paramyosin/102; chitinase/61]	Yes [chitinase resembles a protein from *Dermatophagoides farinae* (mite); paramyosin resembles a protein *Dermatophagoides pteronyssinus* (mite)]
Zuo et al., 2015	China	pupa	Immunoblotting and Western blotting of pupae extract with sera from BM sensitive patients; MALDI-TOF MS and MALDI-TOF MS/MS for protein identification; Bom m 9 expression in bacteria (unnamed)	[vitellogenin/203; chitinase/61; chymotrypsin inhibitor/43; AGAP008849PA/35; 30 kDa lipoprotein (21G1 precursor)/30.3; **Bom m 9** (30 kDa protein precursor)/29.8; 30 kDa lipoprotein (19G1 precursor)/29.6; mature 30 kDa lipoprotein/28.5; Triosephosphate isomerase/26.9; Heat shock protein/20.8;	No [However, Bom m9 has high similarity with the microvitellogenin of the moth *Manduca sexta*]

* molecular weights with bold type were those proteins that, in the study, were present in>10 patients.
[1] The proteins here mentioned are those peculiar of B. mori, marked with a "+" from Table 2 in the work ofBarre et al, 2021.

None of the studies investigated the presence of foodborne viruses. The possibility that foodborne viruses such as Hepatitis A virus (HAV), Hepatitis E virus (HEV) or Norovirus (NoV) could be present in raw insects or insect-based food was proposed by the EFSA (EFSA, 2015). To date, no hazard of viral origins relevant for food safety were identified in the studied farmed insect species (*T. molitor, A. domesticus, Gryllodes sigillatus*, and *A. diaperinus*) (Vandeweyer et al., 2020). However, extending the virus research to other insect species intended for food production is highly recommended, since viruses can be passively transferred from feedstock through residual insect gut contents (EFSA, 2015; Errico et al., 2022). Most viruses in insects are species-specific (not dangerous for humans or other vertebrates), so the major concern is production loss for insect farmers (EFSA, 2015; Eilenberg et al., 2015).

Other microbial hazards can be controlled by adopting appropriate food hygiene practices along the whole production chain and respecting the obligations under the European regulation concerning the marketed forms of insects in Europe. To date, the Regulation (EU) No. 2017/2470 (European Commission (EU), 2017) allowed the trade of insect-based

Table 14
Studies of thermal treatment on the allergenicity of B. mori.

Reference	Year of Study	Country	Life cycle	Treated matrix	Treatment (Temperature value)	Time	Allergenicity evaluation, following treatment	Results
He et al., 2021	not mentioned	China	Pupa	Silkworm Pupae Protein Extract	Dry bath (20 °C; 40 °C; 60 °C; 80 °C; 100 °C) Autoclaving (120 °C)	20 min for each temperature	SDS-page; Western blotting; ELISA	At 120 °C proteins were almost completely denatured. However, 25 to 33 kDa proteins showed heat resistance to temperatures below 100 °C
					Dry bath (60 °C; 80 °C; 100 °C)	5; 10; 20; 30 min for each temperature		Temperatures over 80 °C decreased the proteins' allergenicity, especially after 20–30 min of treatment. However, 25 and 33 kDa proteins had strong IgE binding after 30 min at 100 °C.
Jeong et al., 2016	not mentioned	South Korea	Pupa	Silkworm Pupae Protein Extract	Boiling (temperature not mentioned)	5 min	Immunoblotting	Heat increased the allergenicity of a 27 kDa glycoprotein, along with IgE reactivity to high molecular weight proteins (above 100 kDA). The latter, however, were not characterized through proteome analysis.

products only after thermal drying treatment, either whole or in the form of a powder. These treatments can further reduce the microbiological risk related to the microorganisms hosted by the raw insect but may not be sufficient to reduce the contamination by spore-forming bacteria.

Regarding chemical hazard, the currently available evidence suggests that toxicity is unlikely and that, despite the presence of heavy metal is possible if mulberries are grown on contaminated soil, the heavy metal bioaccumulation is low and manageable. Concentrations of heavy metals in insects depend on the characteristics of the elements and their concentrations in the substrates, the insect species, and their growth stage (EFSA, 2015; Errico et al., 2022). Bioaccumulation of heavy metals has also been described for other insects species approved as food sources in Europe, as for example selenium, As, and Cd in *T. molitor* (van der Fels-Klerx et al., 2018; van der Spiegel et al., 2013; Vijver et al., 2003). In the lack of legislative criteria at EU level on heavy metals in BM, we can refer to limits set up for other species recently authorized according to the EU Novel food regulation (European Commission (EU), 2015). According to these, Pb can reach level as high as 0.075 mg/Kg and Cd as high as 0.1 mg/Kg (Reg. 2017/2470) (European Commission (EU), 2017). Most of insects analysed exceed such levels, however it should be considered that retrieved studies where carried out to evaluate the bioremediation potential of silkworm, thus mulberry fed to insects was grown on artificially contaminated soil. The interesting data is about bioaccumulation potential in the leaf to silkworm path and, as regards Pb and Cd, the majority of retrieved studies do not support such possibility.

Few research studies were retrieved considering toxicity potential, however such evidences even if limited, sum up with the tradition of consumption of BM in several area of the world.

However, it should be acknowledged that few data are available about the chemical hazards deriving from BM consumption as food.

Allergic reactions are likely in sensitive individuals following the ingestion of edible BM, with a particular relevance for people with allergy to crustaceans, mites, and/or molluscs. This evidence is consistent with that reported for other insect species (van der Fels-Klerx et al., 2018), including those species which have obtained positive opinions for human consumption as Novel food by EFSA (Turck et al., 2021a; Turck et al. 2021b). In this cases it is mandatory that label of insects based products report a statement about possible allergic reactions ino consumers with known allergies to crustaceans, molluscs and dust mites (European Commission (EU), 2017). Particular attention must be paid to allergenic proteins contained in BM, as these are the target to understand the origin of sensitization following allergic reaction in people consuming BM as a food source. To date, five BM allergenic proteins have been registered by the WHO/IUIS Allergen Nomenclature Sub-Committee (https://allergen.org/), namely: Bom m 1 (arginine kinase), Bomb m 3 (tropomyosin), Bom m 4 (30 kDa hemolymph lipoprotein), Bom m 5 (30 kDa lipoprotein) and Bomb m 6 (hemolymph lipoprotein 3). BM tropomyosin has similar IgE activity as that from shellfish, as demonstrated in BM sensitive patients by Jeong and colleagues (2017). BM arginine kinase cross-reacts with its analogue from *P. americana* (Liu et al., 2009), a species that is already exploited both as food for humans and feed for livestock in China (Ukoroije et al., 2020). Finally, hemolymph proteins such as the hemocyanin from shrimps behaves as a heat-stable allergen, cross-reacting with its homolog in house dust mite (Gianazza et al., 2021). This is in line with what Barre and colleagues (2021) reported in their work regarding the possibility to consider BM allergenic proteins as pan-allergens. Furthermore, this shrimp protein is also heat-stable, as the 27 kDa hemolymph glycoprotein from BM which had an increased IgE reactivity after being boiled (Jeong et al., 2016).

4.1. Limitations

Considering our objective in investigating BM from a food safety perspective, the greatest limitation of the microbiology results is due to the low number of studies conducted on this insect-based food. Differences in the microbial communities present in/on silkworm pupae and larvae (Kannan et al., 2016) can also influence the microbial characteristics of the final product. In the present review, only three studies analyzed the pupa stage in insect-based food, but there are not enough data to speculate on the impact of the lifecycle stage on the derivative product.

The evidence for chemical hazards of BM when used as a food source, which is available in the literature and collected in the present work, is limited. Few studies have been designed to specifically address dietary exposure, so the majority of evidence originates from studies performed for different purposes. For example, data on the bioaccumulation potential of BM were retrieved from studies dealing with soil bioremediation.

Another important limitation is that we considered only scientific literature published in three Western languages. As the majority of BM studies are conducted in Asian countries it is reasonable to think that we have missed that portion of the literature published in other languages.

4.2. Implications of the results for practice and future research

The introduction of BM and derived products in the human diet or their use as feed is an interesting possibility; however, more studies need

to be performed to fully understand some safety aspects, since to date, a limited number of studies are available.

Future studies dealing with microbial hazards should focus on the detection of foodborne viruses and on the research and quantification of those foodborne pathogenic genera detected by MG studies, such as *Bacillus* spp. (e.g. *B. cereus*), *Clostridium* spp., *Pseudomonas* spp., and *E. coli*. In addition, the lack of specific food safety and process hygiene criteria (European Commission (EC), 2005) for silkworm-based food makes the evaluation of data difficult.

Studies on chemical contamination should be designed to directly investigate BM intended for food production and also as derived products. To date, the only possible evaluations are based on data obtained for different purposes.

Allergic reactions are likely in some people following the ingestion of edible BM. However, to date, too few reports are available in the scientific literature, especially in English. Moreover, underreporting may greatly contribute to this data paucity, so in-depth studies are needed, not just reports on patients. Overall, more data regarding allergenicity reports following ingestion of silkworms are needed. Large cross-sectional surveys across the population must be conducted, both to investigate culinary preferences in Western countries and to determine country-wide prevalences of BM-sensitive individuals. Finally, more studies regarding thermal processing on BM allergenicity are probably required, since this species could constitute a valid food source in the future, and it has especial growth potential in Western countries with a wide population of potential consumers never exposed to this food before.

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Marzoli Filippo: Conceptualization, Writing – original draft, Writing – review & editing. **Antonelli Pietro:** Conceptualization, Writing – original draft, Writing – review & editing. **Saviane Alessio:** Writing – review & editing. **Tassoni Luca:** Writing – review & editing. **Cappellozza Silvia:** Conceptualization, Funding acquisition, Writing – review & editing. **Belluco Simone:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111679.

References

Ahmad, S., Hasan, M. U. U., Sageer, M., & Sahi, S. T. T. (2020). Impact of Bio-Accumulated Cadmium in *Bombyx mori* L. Reared on Mulberry Plants Grown in Heavy Metal-Impregnated Soils in Faisalabad, Pakistan. *African Entomology, 28*(2), 406–414. https://doi.org/10.4001/003.028.0406

Araujo, L. M. L., Souza, C., Zanchin, N. I. T., & Rosário Filho, N. A. (2020). Identification of the major allergenic proteins from silkworm moth (*Bombyx mori*) involved in respiratory allergic diseases. *Allergologia et Immunopathologia, 48*(6), 597–602. https://doi.org/10.1016/j.aller.2019.12.003

Ashfaq, M., Afzal, W., & Hanif, M. A. (2010). Effect of Zn(II) deposition in soil on mulberry-silk worm food chain. *African Journal of Biotechnology, 9*(11), 1665–1672. https://doi.org/10.5897/ajb10.1441

Ashfaq, M., Ahmad, S., Sagheer, M., Hanif, M. A., Abbas, S. K., & Yasir, M. (2012). Bioaccumulation of chromium (III) in silkworm (*Bombyx mori* L.) in relation to mulberry, soil and wastewater metal concentrations. *Journal of Animal and Plant Sciences, 22*(3), 627–634.

Ashfaq, M., Ali, S., & Hanif, M. A. (2009b). Bioaccumulation of cobalt in silkworm (*Bombyx mori* L.) in relation to mulberry, soil and wastewater metal concentrations. *Process Biochemistry, 44*(10), 1179–1184. https://doi.org/10.1016/j.procbio.2009.05.006

Ashfaq, M., Khan, M. I., & Hanif, M. A. (2009a). Use of *Morus alba-Bombyx mori* as a useful template to assess Pb entrance in the food chain from wastewater. *Environmental Entomology, 38*(4), 1276–1282. https://doi.org/10.1603/022.038.0439

Aydoğan, Z. (2021). Anthropo-entomophagy: Quantitatively chemical assessment of some edible arthropods, bought from an e-shop. *Environmental Science and Pollution Research, 28*(12), 15462–15470. https://doi.org/10.1007/s11356-020-11768-y

Ayoade, F., Oyejide, N. E., & Fayemi, S. O. (2014). Isolation, identification, antibiogram and characterization of bacterial pathogens of the silkworm, *Bombyx mori* in south-west Nigeria. *Journal of Biological Sciences, 14*(6), 425–430. https://doi.org/10.3923/jbs.2014.425.430

Barre, A., Pichereaux, C., Simplicien, M., Burlet-Schiltz, O., Benoist, H., & Rougé, P. (2021). A proteomic-and bioinformatic-based identification of specific allergens from edible insects: Probes for future detection as food ingredients. *Foods, 10*(2). https://doi.org/10.3390/foods10020280

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G., & Ricci, A. (2013). Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review. *Comprehensive Reviews in Food Science and Food Safety, 12*(3), 296–313. https://doi.org/10.1111/1541-4337.12014

Bhattacharyya, P., Jha, S., Mandal, P., & Ghosh, A. (2016). Artificial Diet Based Silkworm Rearing System-A Review. *International Journal of Pure & Applied Bioscience, 4*(6), 114–122. https://doi.org/10.18782/2320-7051.2402.

Cappellozza, S., Saviane, A., Tettamanti, G., Squadrin, M., Vendramin, E., Paolucci, P., Franzetti, E., & Squartini, A. (2011). Identification of *Enterococcus mundtii* as a pathogenic agent involved in the “flacherie” disease in *Bombyx mori* L. larvae reared on artificial diet. *Journal of Invertebrate Pathology, 106*(3), 386–393. https://doi.org/10.1016/j.jip.2010.12.007

Chen, B., Du, K., Sun, C., Vimalanathan, A., Liang, X., Li, Y., … Shao, Y. (2018a). Gut bacterial and fungal communities of the domesticated silkworm (*Bombyx mori*) and wild mulberry-feeding relatives. *ISME Journal, 12*(9), 2252–2262. https://doi.org/10.1038/s41396-018-0174-1

Chen, B., Yu, T., Xie, S., Du, K., Liang, X., Lan, Y., … Shao, Y. (2018b). Data descriptor: Comparative shotgun metagenomic data of the silkworm *Bombyx mori* gut microbiome. *Scientific Data, 5*(1), 1–10. https://doi.org/10.1038/sdata.2018.285

Chomchai, S., & Chomchai, C. (2018). Histamine poisoning from insect consumption: An outbreak investigation from Thailand. *Clinical Toxicology, 56*(2), 126–131. https://doi.org/10.1080/15563650.2017.1349320

de Gier, S., & Verhoeckx, K. (2018). Insect (food) allergy and allergens. *Molecular Immunology, 100*(May), 82–106. https://doi.org/10.1016/j.molimm.2018.03.015

Devkota, B., & Schmidt, G. H. (2000). Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. *Agriculture, Ecosystems & Environment, 78*(1), 85–91. https://doi.org/10.1016/S0167-8809(99)00110-3

Dietrich, J., Calabria, C., Hagan, L., Whisman, B., & Johnson, T. (2008). Anaphylaxis to silkworm pupae. *Annals of Allergy Asthma & Immunology*, *100*(1, 1), A75–A75.

Dong, H. L., Zhang, S. X., Chen, Z. H., Tao, H., Li, X., Qiu, J. F., Cuii, W. Z., Sima, Y. H., Cui, W. Z., & Xu, S. Q. (2018). Differences in gut microbiota between silkworms (*Bombyx mori*) reared on fresh mulberry (*Morus alba* var. multicaulis) leaves or an artificial diet. *RSC. Advances, 8*(46), 26188–26200. https://doi.org/10.1039/c8ra04627a

EFSA. (2015). Risk profile related to production and consumption of insects as food and feed. *EFSA Journal, 13*(10). https://doi.org/10.2903/J.EFSA.2015.4257

Eilenberg, J., Vlak, J. M., Nielsen-LeRoux, C., Cappellozza, S., & Jensen, A. B. (2015). Diseases in insects produced for food and feed. *Journal of Insects as Food and Feed, 1*(2), 87–102. https://doi.org/10.3920/JIFF2014.0022

Errico, S., Spagnoletta, A., Verardi, A., Moliterni, S., Dimatteo, S., & Sangiorgio, P. (2022). *Tenebrio molitor* as a source of interesting natural compounds, their recovery processes, biological effects, and safety aspects. *Comprehensive Reviews in Food Science and Food Safety, 21*(1), 148–197. https://doi.org/10.1111/1541-4337.12863

European Commission (EU), (2017). Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. *Official Journal of the European Union L 351*.

European Commission (EU), (2015). Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 *Official Journal of the European Union L 327*.

European Commission (EC), (2005). Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union L 338*.

FAO (2013). *Edible Insects - Future prospects for food and feed security*.

Fasolato, L., Cardazzo, B., Carraro, L., Fontana, F., Novelli, E., & Balzan, S. (2018). Edible processed insects from e-commerce: Food safety with a focus on the Bacillus cereus group. *Food Microbiology, 76*(December 2017), 296–303. https://doi.org/10.1016/j.fm.2018.06.008.

Fei, C., Lu, X. M., Qian, Y. H., Zhang, H., & Mahmood, Q. (2006). Identification of *Enterococcus* sp. from midgut of silkworm based on biochemical and 16S rDNA sequencing analysis. *Annals of Microbiology, 56*(3), 201–205. https://doi.org/10.1007/BF03175006

Feng, W., Wang, X. Q., Zhou, W., Liu, G. Y., & Wan, Y. J. (2011). Isolation and characterization of lipase-producing bacteria in the intestine of the silkworm, *Bombyx mori*, reared on different forage. *Journal of Insect Science, 11*(1), 1536–2442. https://doi.org/10.1673/031.011.13501

Feng, R., Zhu, Q., Xu, Y., Li, W., Ding, Y., Han, L., Rensing, C., & Wang, R. (2019). Safe utilization of polluted soil by arsenic, cadmium and lead through an integrated sericultural measure. *Science of the Total Environment, 659*, 1234–1241. https://doi.org/10.1016/j.scitotenv.2018.12.438

Gautreau, M., Restuccia, M., Senser, K., & Weisberg, S. N. (2017). Familial Anaphylaxis after Silkworm Ingestion. *Prehospital Emergency Care, 21*(1), 83–85. https://doi.org/10.1080/10903127.2016.1204035

Gaylor, M. O., Harvey, E., & Hale, R. C. (2012). House crickets can accumulate polybrominated diphenyl ethers (PBDEs) directly from polyurethane foam common in consumer products. *Chemosphere, 86*(5), 500–505. https://doi.org/10.1016/J.CHEMOSPHERE.2011.10.014

Gianazza, E., Eberini, I., Palazzolo, L., & Miller, I. (2021). Hemolymph proteins: An overview across marine arthropods and molluscs. *Journal of Proteomics, 245*, Article 104294. https://doi.org/10.1016/J.JPROT.2021.104294

Grabowski, N. T., & Klein, G. (2017a). Microbiology of processed edible insect products – Results of a preliminary survey. *International Journal of Food Microbiology, 243*, 103–107. https://doi.org/10.1016/j.ijfoodmicro.2016.11.005

Grabowski, N. T., & Klein, G. (2017b). Microbiological analysis of raw edible insects. *Journal of Insects as Food and Feed, 3*(1), 7–14. https://doi.org/10.3920/JIFF2016.0004

Green, K., Broome, L., Heinze, D., & Johnston, S. (2001). Long distance transport of arsenic by migrating Bogong moths from agricultural lowlands to mountain ecosystems. *Victorian Naturalist, 118*, 112–116.

Hanboonsong, Y., Jamjanya, T., & Durst, P. B. (2013). Six-legged livestock: edible insect farming, collec on and marke ng in Thailand. *Food And Agriculture Organization Of The United Nations Regional Office For Asia And The Pacific Bangkok 2013*.

Handley, M. A., Hall, C., Sanford, E., Diaz, E., Gonzalez-Mendez, E., Drace, K., Wilson, R., Villalobos, M., & Croughan, M. (2007). Globalization, Binational Communities, and Imported Food Risks: Results of an Outbreak Investigation of Lead Poisoning in Monterey County. *California. American Journal of Public Health, 97*(5), 900–906. https://doi.org/10.2105/AJPH.2005.074138

Hayes, B. J., Lewin, H. A., & Goddard, M. E. (2013). The future of livestock breeding: Genomic selection for efficiency, reduced emissions intensity, and adaptation. *Trends in Genetics, 29*(4), 206–214. https://doi.org/10.1016/J.TIG.2012.11.009

He, W., He, K., Sun, F., Mu, L., Liao, S., Li, Q., Yi, J., Liu, Z., & Wu, X. (2021). Effect of heat, enzymatic hydrolysis and acid-alkali treatment on the allergenicity of silkworm pupa protein extract. *Food Chemistry*, *343*(May 2020), 128461. https://doi.org/10.1016/j.foodchem.2020.128461.

Heo, H. S., Choi, J. H., Oh, J. J., Lee, W. J., Kim, S. S., Lee, D. H., Lee, H. K., Song, S. W., Kim, K. H., Choi, Y. K., Ryu, K. S., & Kang, B. H. (2013). Evaluation of general toxicity and genotoxicity of the silkworm extract powder. *Toxicological Research, 29*(4), 263–278. https://doi.org/10.5487/TR.2013.29.4.263

Hou, C., Shi, Y., Wang, H., Li, R., Nartey, M. A., & Guo, X. (2018). Composition and diversity analysis of intestinal microbiota in the fifth instar silkworm. *Bombyx mori L. Invertebrate Survival Journal, 15*(1), 223–233. https://doi.org/10.25431/1824-307X/ISJ.V15I1.223-233.

Hunter, B. A., Hunter, L. M., Johnson, M. S., & Thompson, D. J. (1987). Dynamics of metal accumulation in the grasshopper *Chorthippus brunneus* in contaminated grasslands. *Archives of Environmental Contamination and Toxicology, 16*(6), 711–716. https://doi.org/10.1007/BF01055421

Jamil, K., & Hussain, S. (1992). Biotransfer of metals to the insect *Neochetina eichhornae* via aquatic plants. *Archives of Environmental Contamination and Toxicology, 22*(4), 459–463. https://doi.org/10.1007/BF00212568

Jeong, K. Y., Son, M., Lee, J. Y., Park, K. H., Lee, J. H., & Park, J. W. (2016). Allergenic characterization of 27-kDa glycoprotein, a novel heat stable allergen, from the pupa of silkworm, *Bombyx mori*. *Journal of Korean Medical Science, 31*(1), 18–24. https://doi.org/10.3346/jkms.2016.31.1.18

Jeong, K. Y., Han, I. S., Lee, J. Y., Park, K. H., Lee, J. H., & Park, J. W. (2017). Role of tropomyosin in silkworm allergy. *Molecular Medicine Reports, 15*(5), 3264–3270. https://doi.org/10.3892/mmr.2017.6373

Ji, K., Chen, J., Li, M., Liu, Z., Wang, C., Zhan, Z., Wu, X., & Xia, Q. (2009). *Anaphylactic shock and lethal anaphylaxis caused by food consumption in China* (Vol. 20(5,, 227–231. https://doi.org/10.1016/j.tifs.2009.02.004

Jiang, Y., Jiang, S., Yan, X., Qin, Z., Jia, C., Li, Z., Zhang, J., & Huang, R. (2020). The mobility of cadmium and lead in the soil-mulberry-silkworm system. *Chemosphere, 242*, Article 125179. https://doi.org/10.1016/j.chemosphere.2019.125179

Jiang, L., Wang, Y., Guo, H., Sun, Q., Xie, E., Liuli, H., Li, Q., & Xia, Q. (2021). Toxicological evaluation of transgenic silkworms. *Toxicology Research, 9*(6), 846–854. https://doi.org/10.1093/TOXRES/TFAA089

Kalpana, S., Hatha, A. A. M., & Lakshmanaperumalsamy, P. (1994). Gut microflora of the larva of silkworm, *Bombyx mori*. *International Journal of Tropical Insect Science, 15*(4–5), 499–502. https://doi.org/10.1017/s1742758400015873

Kannan, M., Suryaaathmanathan, V., Saravanakumar, M., Jaleel, A., Romanelli, D., Tettamanti, G., & Krishnan, M. (2016). Proteomic analysis of the silkworm midgut during larval-pupal transition. *Invertebrate Survival Journal, 13*(1), 191–204. https://doi.org/10.25431/1824-307X/ISJ.V13I1.191-204.

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., & Nout, M. J. R. (2012). Microbiological aspects of processing and storage of edible insects. *Food Control, 26*(2), 628–631. https://doi.org/10.1016/J.FOODCONT.2012.02.013

Komase, Y., Sakata, M., Azuma, T., Tanaka, A., & Nakagawa, T. (1997). IgE antibodies against midge and moth found in Japanese asthmatic subjects and comparison of allergenicity between these insects. *Allergy: European. Journal of Allergy and Clinical Immunology, 52*(1), 75–81. https://doi.org/10.1111/j.1398-9995.1997.tb02548.x

Kurdi, P., Chaowiwat, P., Weston, J., & Hansawasdi, C. (2021). Studies on microbial quality, protein yield, and antioxidant properties of some frozen edible insects. *International Journal of Food Science, 2021*. https://doi.org/10.1155/2021/5580976

Li, G.-N., Xia, X.-J., Zhao, H.-H., Sendegeya, P., & Zhu, Y. (2015). Identification and Characterization of *Bacillus cereus* SW7-1 in *Bombyx mori* (Lepidoptera: Bombycidae). *Journal of Insect Science, 15*(1), 136. https://doi.org/10.1093/JISESA/IEV121

Li, G.-N., Xia, X.-J., Tang, W.-C., & Zhu, Y. (2016). Intestinal microecology associated with fluoride resistance capability of the silkworm (*Bombyx mori* L.). *Applied Microbiology and Biotechnology 2016 100:15*, *100*(15), 6715–6724. https://doi.org/10.1007/S00253-016-7480-1.

Li, F., Li, M., Mao, T., Wang, H., Chen, J., Lu, Z., Qu, J., Fang, Y., Gu, Z., & Li, B. (2020). Effects of phoxim exposure on gut microbial composition in the silkworm. *Bombyx mori. Ecotoxicology and Environmental Safety, 189*, Article 110011. https://doi.org/10.1016/j.ecoenv.2019.110011

Liang, Xue, Fu, Y., Tong, L., & Liu, H. (2014). Microbial shifts of the silkworm larval gut in response to lettuce leaf feeding. *Applied Microbiology and Biotechnology 2014 98:8*, *98*(8), 3769–3776. https://doi.org/10.1007/S00253-014-5532-Y.

Liang, X., Fu, Y., & Liu, H. (2015). Isolation and characterization of enzyme-producing bacteria of the silkworm larval gut in bioregenerative life support system. *Acta Astronautica, 116*, 247–253. https://doi.org/10.1016/J.ACTAASTRO.2015.07.010

Liang, Xili, Sun, C., Chen, B., Du, K., Yu, T., Luang-In, V., Lu, X., & Shao, Y. (2018). Insect symbionts as valuable grist for the biotechnological mill: an alkaliphilic silkworm gut bacterium for efficient lactic acid production. *Applied Microbiology and Biotechnology 2018 102:11*, *102*(11), 4951–4962. https://doi.org/10.1007/S00253-018-8953-1.

Lindqvist, L. (1992). Accumulation of cadmium, copper, and zinc in five species of phytophagous insects. *Environmental Entomology, 21*(1), 160–163. https://doi.org/10.1093/ee/21.1.160

Ling, X. D., Dong, W. T., Zhang, Y., Hu, J. J., Zhang, W. D., Wu, J. T., Liu, J. X., & Zhao, X. X. (2019). Baculoviral infection reduces the expression of four allergen proteins of silkworm pupa. *Archives of Insect Biochemistry and Physiology, 100*(4). https://doi.org/10.1002/arch.21539

Liu, Z., Xia, L., Wu, Y., Xia, Q., Chen, J., & Roux, K. H. (2009). Identification and characterization of an arginine kinase as a major allergen from silkworm (*Bombyx mori*) larvae. In *International Archives of Allergy and Immunology* (Vol. 150, Issue 1, pp. 8–14). https://doi.org/10.1159/000210375.

Mancini, S., Paci, G., Ciardelli, V., Turchi, B., Pedonese, F., & Fratini, F. (2019). *Listeria monocytogenes* contamination of *Tenebrio molitor* larvae rearing substrate: Preliminary evaluations. *Food Microbiology, 83*, 104–108. https://doi.org/10.1016/j.fm.2019.05.006

Merrington, G., Winder, L., & Green, I. (1997). The uptake of cadmium and zinc by the bird-cherry oat aphid *Rhopalosiphum padi* (Homoptera: Aphididae) feeding on wheat grown on sewage sludge amended agricultural soil. *Environmental Pollution, 96*(1), 111–114. https://doi.org/10.1016/S0269-7491(96)00149-2

Milanović, V., Osimani, A., Pasquini, M., Aquilanti, L., Garofalo, C., Taccari, M., Cardinali, F., Riolo, P., & Clementi, F. (2016). Getting insight into the prevalence of antibiotic resistance genes in specimens of marketed edible insects. *International Journal of Food Microbiology, 227*, 22–28. https://doi.org/10.1016/J.IJFOODMICRO.2016.03.018

Mohanta, M. K., Saha, A. K., Saleh, D. K. M. A., Islam, M. S., Mannan, K. S. B., & Fakruddin, M. (2015). Characterization of *Klebsiella granulomatis* pathogenic to silkworm, *Bombyx mori* L. *3. Biotech, 5*(4), 577–583. https://doi.org/10.1007/s13205-014-0255-4

NVWA, (Netherlands Food and Consumer Product Safety Authority). (2014). *Advisory report on the risks associated with the consumption of mass-reared insects*.

Osimani, A., Garofalo, C., Milanović, V., Taccari, M., Cardinali, F., Aquilanti, L., Pasquini, M., Mozzon, M., Raffaelli, N., Ruschioni, S., Riolo, P., Isidoro, N., & Clementi, F. (2017). Insight into the proximate composition and microbial diversity of edible insects marketed in the European Union. *European Food Research and Technology, 243*(7), 1157–1171. https://doi.org/10.1007/S00217-016-2828-4

Pradhap, M., & Selvisabhanayakam. (2011). Identification and molecular characterization of *Vibrio cholerae* from the midgut of silkworm *Bombyx mori* (Linn.) Lepidoptera; bombycidae. *International Journal of Pharma and Bio Sciences*, *2*(4), 29–37.

Osimani, A., Milanović, V., Cardinali, F., Roncolini, A., Garofalo, C., Clementi, F., … Aquilanti, L. (2018b). Bread enriched with cricket powder (*Acheta domesticus*): A technological, microbiological and nutritional evaluation. *Innovative Food Science and Emerging Technologies, 48*, 150–163. https://doi.org/10.1016/j.ifset.2018.06.007

Osimani, A., Milanović, V., Garofalo, C., Cardinali, F., Roncolini, A., Sabbatini, R., … Aquilanti, L. (2018a). Revealing the microbiota of marketed edible insects through PCR-DGGE, metagenomic sequencing and real-time PCR. *International Journal of Food Microbiology, 276*, 54–62. https://doi.org/10.1016/j.ijfoodmicro.2018.04.013

Prem Anand, A. A., Vennison, S. J., Sankar, S. G., Gilwax Prabhu, D. I., Vasan, P. T., Raghuraman, T., Jerome Geoffrey, C., & Vendan, S. E. (2010). Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. *Journal of Insect Science, 10*(1), 107. https://doi.org/10.1673/031.010.10701

Prince, S. P. M., Senthilkumar, P., & Subburam, V. (2001). Mulberry-silkworm food chain-A templet to assess heavy metal mobility in terrestrial ecosystems. *Environmental Monitoring and Assessment, 69*(3), 231–238. https://doi.org/10.1023/A:1010715606097

Priyadharshini, P., Mahalingam, C. A., & Shashidhar, K. R. (2008). Identification and characterization of bacterial pathogens in silkworm. *Bombyx mori L. Current Biotica, 2*(2), 181–192.

Rattana, S., Katisart, T., Sungthong, B., & Butiman, C. (2017). Acute and sub-acute toxicities of Thai silkworm powder (*Bombyx mori* linn.) from three races in male wistar rats and in vitro antioxidant activities. *Pharmacognosy Journal, 9*(4), 541–545. https://doi.org/10.5530/pj.2017.4.87

Sakthivel, S., Angaleswari, C., & Mahalingam, P. U. (2012). Isolation and identification of bacteria responsible for flacherie in silkworms. *Advances in Applied Science Researcg, 3*(6), 4066–4068.

Schirone, M., & Visciano, P. (2021). Trends of Major Foodborne Outbreaks in the European Union during the Years 2015–2019. *Hygiene 2021, Vol. 1, Pages 106-119, 1*(3), 106–119. https://doi.org/10.3390/HYGIENE1030010.

Si, L., Zhang, J., Hussain, A., Qiao, Y., Zhou, J., & Wang, X. (2021). Accumulation and translocation of food chain in soil-mulberry (*Morus alba* L.)-silkworm (*Bombyx mori*) under single and combined stress of lead and cadmium. *Ecotoxicology and Environmental Safety, 208*, Article 111582. https://doi.org/10.1016/j.ecoenv.2020.111582

Stoops, J., Crauwels, S., Waud, M., Claes, J., Lievens, B., & Van Campenhout, L. (2016). Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria migratorioides*) sold for human consumption. *Food Microbiology, 53*, 122–127. https://doi.org/10.1016/j.fm.2015.09.010

Subramanian, S., Gadhave, K. R., Mohanraj, P., & Thangamalar, A. (2009). Use of 16S rRNA probes for characterization of gut microflora of silkworm (*Bombyx mori* L.) breeds The role of bacterial endosymbionts in shaping the insect-virus relationship in *Bemisia tabaci*. View project Use of 16S rRNA probes for charact. *Karnataka. J. Agric. Sci, 22*(3), 476–478.

Sun, Z., Lu, Y., Zhang, H., Kumar, D., Liu, B., Gong, Y., Zhu, M., Zhu, L., Liang, Z., Kuang, S., Chen, F., Hu, X., Cao, G., Xue, R., & Gong, C. (2016). Effects of BmCPV infection on silkworm *Bombyx mori* intestinal bacteria. *PLoS ONE, 11*(1), Article e0146313. https://doi.org/10.1371/journal.pone.0146313

Sun, Z., Kumar, D., Cao, G., Zhu, L., Liu, B., Zhu, M., Liang, Z., Kuang, S., Chen, F., Feng, Y., Hu, X., Xue, R., & Gong, C. (2017). Effects of transient high temperature treatment on the intestinal flora of the silkworm *Bombyx mori. Scientific Reports, 7*(1), 1–15. https://doi.org/10.1038/s41598-017-03565-4

Suzuki, K. T., Aoki, Y., Nishikawa, M., Masui, H., & Matsubara, F. (1984). Effect of cadmium-feeding on tissue concentrations of elements in germ-free silkworm (*Bombyx mori*) larvae and distribution of cadmium in the alimentary canal. *Comparative Biochemistry and Physiology. Part C, Comparative, 79*(2), 249–253. https://doi.org/10.1016/0742-8413(84)90194-4

Tao, H. P., Shen, Z. Y., Zhu, F., Xu, X. F., Tang, X. D., & Xu, L. (2011). Isolation and identification of a pathogen of silkworm *Bombyx mori*. *Current Microbiology, 62*(3), 876–883. https://doi.org/10.1007/s00284-010-9796-x

Thomas, J., Brunton, J., & Graziosi, S. (2010). *EPPI-Reviewer 4: software for research synthesis.* EPPI-Centre Software.

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., … Knutsen, H. K. (2021a). Safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *19*(1). https://doi.org/10.2903/J.EFSA.2021.6343.

Turck, D., Castenmiller, J., De Henauw, S., Hirsch-Ernst, K. I., Kearney, J., Maciuk, A., Mangelsdorf, I., McArdle, H. J., Naska, A., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel, T., Heinonen, M., Marchelli, R., … Knutsen, H. K. (2021b). Safety of frozen and dried formulations from migratory locust (*Locusta migratoria*) as a Novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*, *19*(7). https://doi.org/10.2903/j.efsa.2021.6667.

Ukoroije, R., Bawo, D., Boate, R., & Suotonye, D. (2020). Cockroach (*Periplaneta americana*): Nutritional Value as Food and Feed For Man and Livestock The efficacy of *Azadirachta indica, Ocimum gratissimum* and *Dracaena arborea* against the household pest *Periplaneta america* View project Cockroach (*Periplaneta americana*): Nutritional Value as Food and Feed For Man and Livestock. *Annals of Forest Science Journal, 56507*, 37–46. https://doi.org/10.9734/afsj/2020/v15i230150

van der Fels-Klerx, H. J., Camenzuli, L., Belluco, S., Meijer, N., & Ricci, A. (2018). Food Safety Issues Related to Uses of Insects for Feeds and Foods. *Comprehensive Reviews in Food Science and Food Safety, 17*(5), 1172–1183. https://doi.org/10.1111/1541-4337.12385

van der Spiegel, M., Noordam, M. Y., & van der Fels-Klerx, H. J. (2013). Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production. *Comprehensive Reviews in Food Science and Food Safety, 12*(6), 662–678. https://doi.org/10.1111/1541-4337.12032

Vandeweyer, D., Crauwels, S., Lievens, B., & Van Campenhout, L. (2017). Microbial counts of mealworm larvae (*Tenebrio molitor*) and crickets (*Acheta domesticus* and *Gryllodes sigillatus*) from different rearing companies and different production batches. *International Journal of Food Microbiology, 242*, 13–18. https://doi.org/10.1016/j.ijfoodmicro.2016.11.007

Vandeweyer, D., Wynants, E., Crauwels, S., Verreth, C., Viaene, N., Claes, J., Lievens, B., & Van Campenhout, L. (2018). Microbial Dynamics during Industrial Rearing, Processing, and Storage of Tropical House Crickets (*Gryllodes sigillatus*) for Human Consumption. *Applied and Environmental Microbiology, 84*(12). https://doi.org/10.1128/AEM.00255-18

Vandeweyer, D., Lievens, B., & Van Campenhout, L. (2020). Identification of bacterial endospores and targeted detection of foodborne viruses in industrially reared insects for food. *Nature Food, 1*(8), 511–516. https://doi.org/10.1038/s43016-020-0120-z

Venkatappa, B., Chandrakala, A. N., Anuradha, P., & Chatrappa, B. (2005). Evaluation of silkmoth (*Bombyx mori*) allergens in sericultue grainage industries as major pollutants. *Journal of Scientific and Industrial Research, 64*(4), 287–292.

Vijver, M., Jager, T., Posthuma, L., & Peijnenburg, W. (2003). Metal uptake from soils and soil-sediment mixtures by larvae of *Tenebrio molitor* (L.) (Coleoptera). *Ecotoxicology and Environmental Safety, 54*(3), 277–289. https://doi.org/10.1016/S0147-6513(02)00027-1

Wan, X., Lei, M., Chen, T., Tan, Y., & Yang, J. (2017). Safe utilization of heavy-metal-contaminated farmland by mulberry tree cultivation and silk production. *Science of the Total Environment, 599–600*, 1867–1873. https://doi.org/10.1016/j.scitotenv.2017.05.150

Wang, H., Hu, W., Liang, Z., Zeng, L., Li, J., Yan, H., Yang, P., Liu, Z., & Wang, L. (2016). Thiol peroxiredoxin, a novel allergen from *Bombyx mori*, modulates functions of macrophages and dendritic cells. *American Journal of Translational Research, 8*(12), 5320–5329.

Xia, Q., Zhou, Z., Lu, C., Cheng, D., Dai, F., Li, B., … Yang, H. (2004). A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). *Science, 306*(5703), 1937–1940. https://doi.org/10.1126/science.1102210

Yang, S.-Y., Han, M.-J., Kang, L.-F., Li, Z.-W., Shen, Y.-H., & Zhang, Z. (2014). Demographic history and gene flow during silkworm domestication. *BMC Evolutionary Biology 2014 14:1*, *14*(1), 1–8. https://doi.org/10.1186/S12862-014-0185-0.

Yeruva, T., Vankadara, S., Ramasamy, S., & Lingaiah, K. (2020). Identification of otential probiotics in the midgut of mulberry silkworm, *Bombyx mori* through metagenomic approach. *Probiotics and Antimicrobial Proteins, 12*(2), 635–640. https://doi.org/10.1007/s12602-019-09580-3

Yigit, S., Hallaj, N. S., Sugarman, J. L., Chong, L. C., Roman, S. E., Abu-Taleb, L. M., Goodman, R. E., Johnson, P. E., & Behrens, A. M. (2021). Toxicological assessment and food allergy of silk fibroin derived from *Bombyx mori* cocoons. *Food and Chemical Toxicology, 151*, Article 112117. https://doi.org/10.1016/j.fct.2021.112117

Zhang, J., Shen, Zhong-yuan, Tang, Xu-dong, Xu, Li, Zhu, Feng, et al. (2013). Isolation and identification of a pathogen, Providencia rettgeri, Bombyx mori. *Journal of Bacteriology Research, 5*(2), 22–28. https://doi.org/10.5897/jbr2012.0109

Zhang, Y., Shang, R., Zhang, J., Li, J., Zhu, G., Yao, M., Sun, J., & Shen, Z. (2020). Isolation and identification of two *Serratia marcescens* strains from silkworm, *Bombyx mori*. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 113*(9), 1313–1321. https://doi.org/10.1007/s10482-020-01442-1

Zhao, X., Li, L., Kuang, Z., Luo, G., & Li, B. (2015). Proteomic and immunological identification of two new allergens from silkworm (*Bombyx mori* L.) pupae. *Central European Journal of Immunology, 40*(1), 30–34. https://doi.org/10.5114/ceji.2015.50830

Zhou, L., Zhao, Y., Wang, S., Han, S., & Liu, J. (2015). Lead in the soil-mulberry (*Morus alba* L.)-silkworm (Bombyx mori) food chain: Translocation and detoxification. *Chemosphere, 128*, 171–177. https://doi.org/10.1016/j.chemosphere.2015.01.031

Zhuang, P., Zou, H., & Shu, W. (2009). Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: Field study. *Journal of Environmental Sciences, 21*(6), 849–853. https://doi.org/10.1016/S1001-0742(08)62351-7

Zuo, J., Lei, M., Yang, R., & Liu, Z. (2015). Bom m 9 from Bombyx mori is a novel protein related to asthma. *Microbiology and Immunology, 59*(7), 410–418. https://doi.org/10.1111/1348-0421.12271