PARMA SUMMER SCHOOL

26 – 28 SEPTEMBER 2023

Innovative food products

Case study: Safety of insects as novel foods

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EUROPEAN FOOD SAFETY AUTHORITY

UNIVERSITÀ DI PARMA

SCHOOL OF ADVANCED STUDIES ON FOOD AND NUTRITION

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Outline

I Introduction

- Who we are & who you are;
- In-depth presentation on Insects risk assessment;
- Break

II Case study

- Critical points of the NF assessment: UV treated powder from *Tenebrio molitor*
- *Q&A*
- Break

III Wrap-up

- Do you know? Quiz with award!

Introductions

We read a curiosity fact and let you guess to which of the us the story belongs to.

About you!

In depth presentation

- Categories and trends novel foods?
- Risk assessment of novel foods.
- Focus on risk assessment of insects.

Risk assessment process

EFSA Scientific Officers
Nutrition & Food Innovation Unit

Working Group on Novel Foods
14 experts

Panel on Nutrition, Novel Foods and Food Allergens
16 experts

Fundamental principles

The novel food shall be safe under the proposed conditions of use

The novel food cannot be nutritionally disadvantageous

The efficacy of the novel food is not assessed

Novel food guidance (2016/21+update)

- Administrative data
- Introduction
- Identity of the novel food
- Production process
- Compositional data
- Specifications
- History of use of the novel food and its source
- Proposed uses and use levels, anticipated intake
- Absorption, distribution, metabolism, excretion (ADME)
- Nutritional information
- Toxicological information
- Allergenicity
- Conclusions

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 21 September 2016

doi: 10.2903/j.efsa.2016.4594

Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), Dominique Turck, Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Marco Vinceti, Peter Willatts, Karl-Heinz Engel, Rosangela Marchelli, Annette Pöting, Morten Poulsen, Seppo Salminen, Josef Schlatter, Davide Arcella, Wolfgang Gelbmann, Agnès de Sesmaisons-Lecarré, Hans Verhagen and Hendrik van Loveren

Abstract

Following the adoption of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, the European Commission requested EFSA to update and develop scientific and technical guidance for the preparation and presentation of applications for authorisation of novel foods. This guidance presents a common format for the organisation of the information to be presented in order to assist the applicant in preparing a well-structured application to demonstrate the safety of the novel food. The application should be comprehensive and complete. This guidance outlined the data needed for the safety assessments of novel foods. Requirements which should be covered in all applications relate to the description of the novel food, production process, compositional data, specification, proposed uses and use levels, and anticipated intake of the novel food. Further sections on the history of use of the novel food and/or its source, absorption, distribution, metabolism, excretion, nutritional information, toxicological information and allergenicity should be considered by the applicant by default. If not covered in the application, this should be justified. The applicant should integrate the data presented in the different sections to provide their overall considerations on how the information supports the safety of the novel food under the proposed conditions of use. Where potential health hazards have been identified, they should be discussed in relation to the anticipated intakes of the novel food and the proposed target populations. On the basis of the information provided, EFSA will assess the safety of the novel food under the proposed conditions of use.

Categories of NFs

- New production process
- New or modified molecular structure
- From microorganisms, fungi or algae
- From plants or their parts
- Vitamins and minerals from new process / nanomaterials
- Of mineral origin
- From animals or their parts
- Cell or tissue cultures derived from the living
- Engineered nanomaterials
- Exclusive use in food supplements prior to May 1997

Trend of NF under risk assessment

Adapted from: Ververis et al. (2020), Novel foods in the European Union: Scientific requirements and challenges of the risk assessment process by the European Food Safety Authority. *Food Research International*, 137, 109515.

Trending categories of NFs

Novel Proteins and/or sources

Insects

Plant-based

Algae

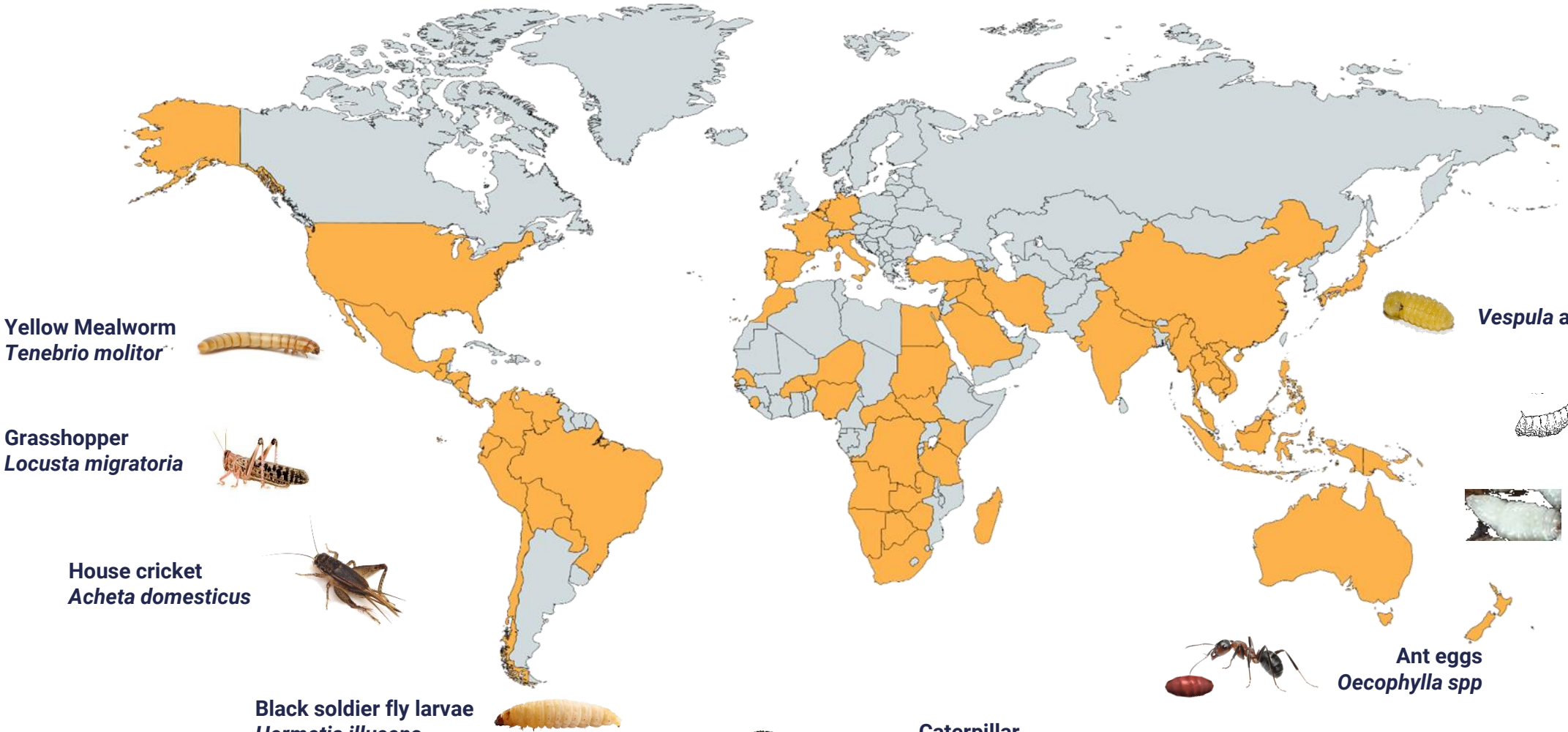
Tissue culture

Fungi

By-products

Insects as food around the world

Between 1600 and 2000 insect species reported to be consumed as food around the globe

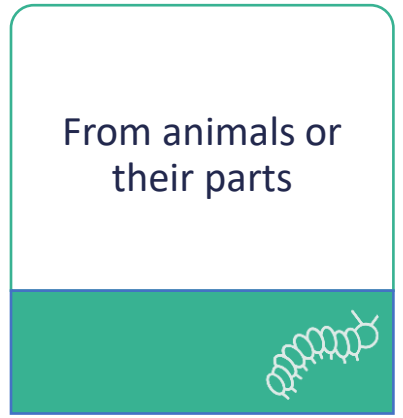


Why insects are Novel Foods in Europe?

Foods or ingredients that have not been used for human consumption to a significant degree in the EU before 15 May 1997

Insects

- New Sources
- New processes & technologies
- Traditional foods (non-EU countries)



Novel Food Category
Regulation (EU) 2015/2283

NF application received by EFSA

6 EFSA Suitability check

6 EFSA Risk assessment

7 EFSA Opinions adopted

8 Adult stage

2+2+1 *Acheta domesticus*
(house crickets)

1+1 *Locusta migratoria*
(grasshoppers)

1 *Gryllodes sigillatus**
(banded crickets)

9 Larval stage

2+2+3 *Tenebrio molitor*
(yellow mealworms)

1+1 *Alphitobius diaperinus*
(lesser mealworms)

1+1 *Hermetia illucens*
(black soldier fly larvae)

1 *Apis mellifera*
(honey bee male drones)

* the applicant withdrew the application during the risk assessment

Production Process: Processing & Forms

(examples from the Novel Food application dossiers received by EFSA)

- **Refrigerated**
  - Thermal treatment
- **Frozen**
  - Thermal treatment (not always)
    - Freezing **(whole)**
    - Grinding **(paste)**
- **Dried**
  - Thermal treatment
    - Drying **(whole)**
    - Grinding **(powder)**
- **UV-treatment**
- **Fat extraction**

Production Process

Insect species

- Physical hazards/risks
- Developmental stage
- Endogenously produced compounds

Farming

- Rearing conditions
- Feeding substrate

Harvest & killing

- Fasting step
- Intestinal track not removed
- Separation of insects from frass, deceased animals

Processing

- Microbiological aspects
- Processing contaminants
- Stability

Characterisation

- Whole insects: **complex foods**
- Qualitative and quantitative characterization of the **main constituents & proximate analysis** (and challenges)
- **Nutritionally relevant** constituents (e.g., vitamins, minerals)

- **substances of possible concern** to human health (inherent, rearing specific, processing)
- Collection & extrapolation of data from **literature**
- **Stability** (microbiological & oxidative stability of fats)

History of use

- Extend of use & Role in the diet
- Precautions and restrictions of use (e.g. removal or parts before preparation and consumption)
- Non-food uses (e.g. medicine)

- **House crickets** (Thailand, Lao, Cambodia, China, Mexico)
- **Lesser mealworms** (no history of use)

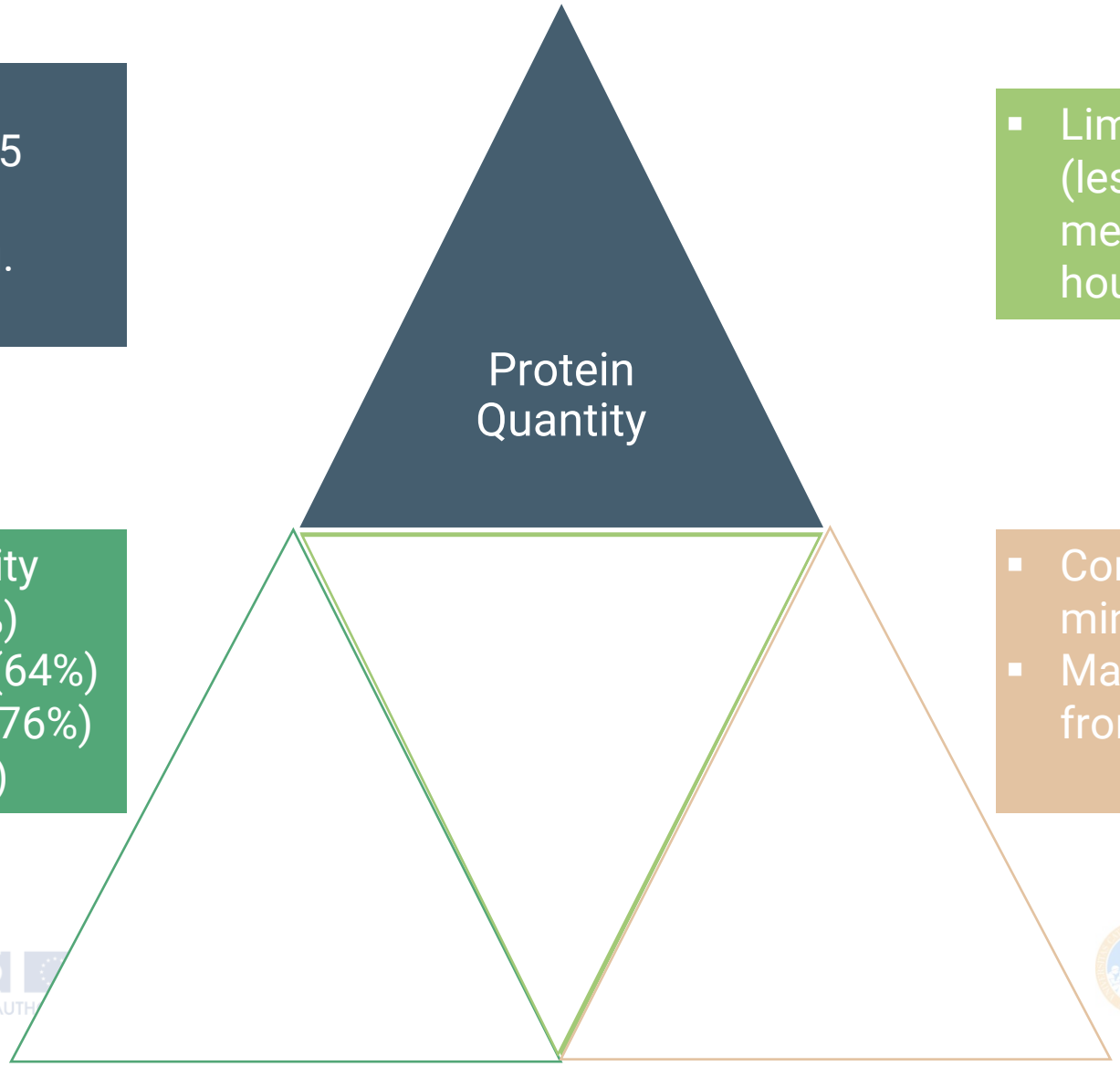
Nutritional information

- **Nutrients** profile
- **Bioavailability** of nutrients, as appropriate
- **Antinutrients** (e.g., tannins, phytic acid, oxalic acid, trypsin inhibitors)

- **Chitin**, a major component of insects' exoskeleton, may bind bivalent minerals (inhibiting thus their absorption);
- **Protein quality** (amino acid profile & protein digestibility)

Nutritional information

- "true" protein can be overestimated, when 6.25 factor is used
- Non-protein nitrogen, e.g. chitin



- Limiting AA: sulphur AA (lesser mealworm, yellow mealworm, grasshopper, house crickets)

true ileal nitrogen digestibility

- Dried house cricket (67%)
- Dried yellow mealworm (64%)
- Dried lesser mealworm (76%)
- Dried Grasshopper (55%)

- Considerable amounts of minerals
- Manganese intake (<5% of Mn from background diet)

Proposed uses and use levels

- Form of use: **whole, dried, powder**
- Examples of food categories to be used as an ingredient: **biscuits, pasta, snacks, soups, yoghurts, meat imitates**
- Maximum **inclusion levels** to be reported

- Anticipated **intake** (mean & 95th percentile)
- **Exposure** assessment (components of concern, minerals, vitamins)

Nutritional information

- are among the **novel protein sources** being explored in our food system;
- are complex organisms, which makes the **compositional characterization challenging;**
- may be high in protein (especially their dried forms), although the true protein levels can be **overestimated** when the substance chitin is present;
- if they entirely replace other protein sources of higher quality, there may be an **impact on protein nutrition** if the overall protein intake is low;
- could trigger **allergic reactions** to certain individuals.

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Innovative food products

Break

10:00

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Breaktime for PowerPoint by Flow Simulation Ltd. Pin controls when stopped

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Case study

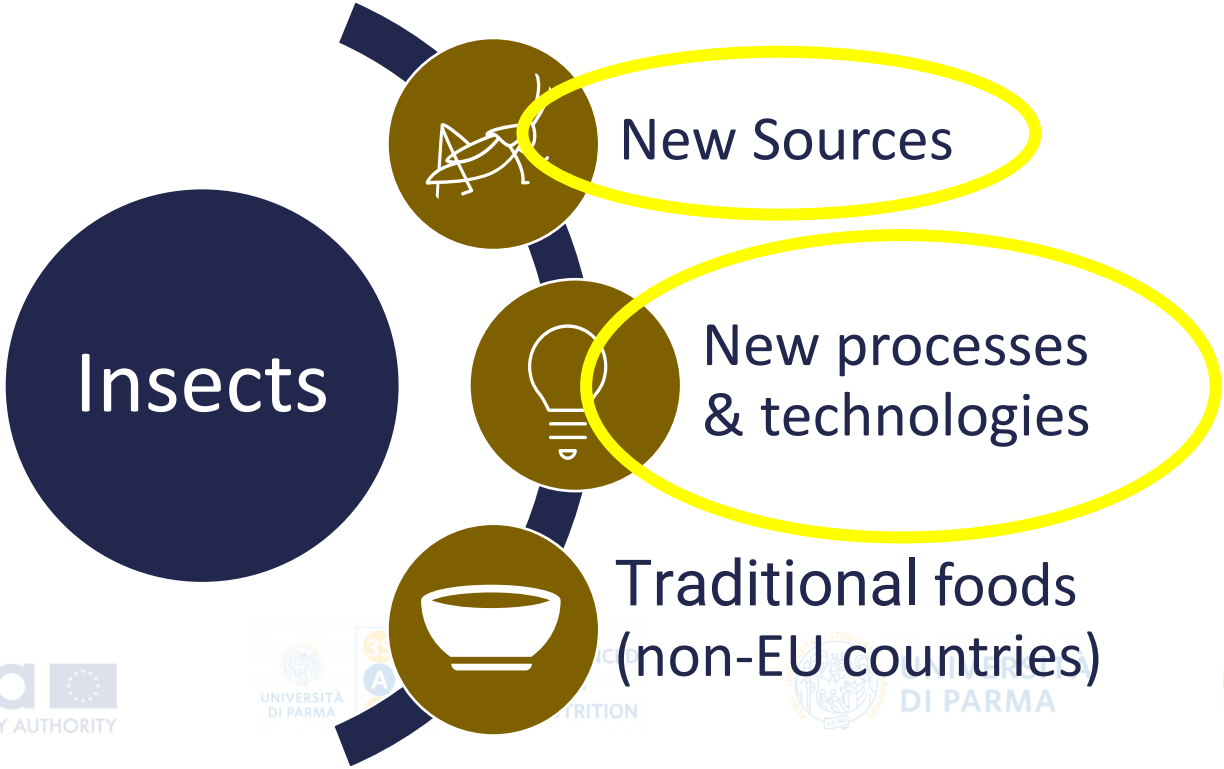
UV-treated powder of whole yellow mealworm

(*Tenebrio molitor* larva)

Case Study

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

- **Adopted by EFSA NDA** Panel on 28 March 2023.
- **Authorization** process by EC & EU Member States: **ongoing**



CASE STUDY

Case Study - Identity

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

- Form (developmental stage): larvae
- Family: Tenebrionidae
- Scientific synonyms: T. molitor Linnaeus
- Common names: yellow mealworm, mealworm, vers de farine
- Geographic area of origin: Eastern Mediterranean
- Initial livestock origin: external supplier & internal breeding
- Confirmation of identity: PCR tests
- Part(s) consumed: whole

3.2. Identity of the NF

The NF is the UV-treated powder of the whole dried yellow mealworm. The term 'mealworm' refers to the larval form of *T. molitor*, an insect species that belongs to the family of Tenebrionidae (darkling beetles). Another identified scientific synonym is *T. molitor* Linnaeus. 'Yellow mealworms', 'mealworms', 'vers de farine' 'tenebrio meunier' and 'mealworm meal' are some of the common names for *T. molitor* larva or products thereof.

The Eastern-Mediterranean region appears to be the point of origin for *T. molitor* sp. (Panagiotakopulu, 2000). However, *T. molitor* sp. is currently present in various regions worldwide, due to colonisation and trade (Panagiotakopulu, 2001). The applicant received the initial livestock of *T. molitor* from an external supplier and proceeded with the farming of the insects. The identity of the insects, both those from the external supplier and those subsequently bred by the applicant, was established using PCR testing.

The whole mealworms are used for the production of the NF. The insects are farmed under controlled rearing conditions.

CASE STUDY

Case Study - Production process

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

3.3. Production process

According to the information provided, the NF is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles. The production process can be divided into three distinctive parts, i.e. farming, harvesting and post-harvest processing.

Farming includes mating of the adult insect population and rearing of the larvae. The eggs are separated from the adult insects and are hatched separately. After being hatched from the eggs, the light yellow-brown larvae grow for 12 weeks in dedicated containers made of high-density polypropylene. This reduces the probability of plastic ingestion by the larvae (EFSA NDA Panel, 2021a, b). The containers are certified for food contact. The applicant stated that no antibiotics or hormones are used during the rearing of the larvae.

Yellow mealworms have the potential to bioaccumulate chemical agents such as heavy metals, pesticide residues and other undesirable compounds (e.g. polychlorinated biphenyls (PCBs), dioxins) through their feed intake (Lindqvist and Block, 1995; Vijver et al., 2003; Bednarska and Świątek, 2016; Houbraken et al., 2016; Van der Fels-Klerx et al., 2016; Ghannem et al., 2018). The applicant reported that the feed administered to the insects is of plant origin (commercially available chicken feed and vegetables that follow the provisions of Regulation (EC) 834/2007[5] and Regulation (EC) 889/2008[6], compliant with Directive 2002/32/EC). The Panel notes that the vitamin D3 level in the feed is at a concentration of 2,750 IU/kg (68.75 μg/kg)[7] and that this level is not compliant with the permitted vitamin D3 level of 2,000 IU/kg in feed for 'other species' of complete feeding stuff with a moisture content of 12% [Commission Implementing Regulation (EU) 2017/1492[8]]. Considering the vitamin D3 values previously reported in dried yellow mealworms (0.989 μg/100 g in EFSA NDA Panel, 2021a; < 0.25 μg/100 g in EFSA NDA Panel, 2021b) and the vitamin D3 levels reported by the applicant in the non-UV-treated yellow mealworm powder (1.86 ± 0.87 μg/100 g) (Table 9), the Panel concludes that the feed does not have a substantial impact on the vitamin D3 levels of the NF.

The applicant informed that the feed substrate used may contain gluten-containing grains and soy-derived ingredients. Water is provided to the larvae through some components of the feed (vegetables).

It has been previously discussed that *T. molitor* can be infected, e.g. by bacteria, parasites, entomopathogenic fungi and viruses, often as a result of poor hygiene farming conditions (EFSA NDA Panel, 2021a,b). However, the Panel concludes that the production process steps implemented, and the specification limits set, mitigate the risk of these biological hazards.

During the rearing of the larvae, deceased insects and faeces are monitored and removed. Two distinct sorting steps are performed, when the larvae are of ~ 6 and of ~ 12 weeks. Mechanical sieving separates the larvae from the substrate, exuvia and faeces. Deceased larvae have a darker colour compared to the alive larvae and are removed via visual inspection. The 6-week-old larvae are further grown, and the 12-week-old larvae are harvested to be processed. After the harvest (removal from the feed substrate), a 24-h fasting step is implemented, to allow the larvae to discard their bowel content. Deceased larvae after the fasting step are removed upon visual inspection.

The post-harvest processing includes the freezing of the larvae (−18° C for 5 mins), with subsequent killing by blanching. Those two steps contribute to the reduction of the microbial load of the larvae as well as to the elimination of potentially present viruses and parasites (Kooh et al., 2019; Vandeweyer et al., 2021). Furthermore, blanching reduces enzymatic activity (e.g. tyrosinase/phenoloxidase) (Janssen et al., 2017a) that otherwise might induce enzymatic browning in the larvae (Nappi and Vass, 1993; Nappi and Ottaviani, 2000; Sugumaran et al., 2000; Nappi and Christensen, 2005; Vigneron et al., 2014). The blanched larvae undergo drying in a ventilated dehydrator (70°C), with the target water activity being < 0.6. The dried larvae are subsequently ground mechanically to produce the insect powder.

The resulting powder is then radiated with UVB light to enhance the concentration of vitamin D3 in the NF. The NF is stored in hermetically closed opaque packaging certified for food contact (laminated aluminium DoyPack), at room temperature (~ 50% relative humidity).

The Panel considers that the production process is sufficiently described.

Main Steps

- **Farming**
  (mating, hatching, rearing, feed)
- **Harvesting**
  (fasting, cleaning)
- **Post-harvest processing**
  (freezing, blanching, drying, grinding, UV radiation, storage)

CASE STUDY

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The Panel considers that the production process is sufficiently described.

Main Steps

- **Farming**
  (mating, hatching, rearing, feed)
- **Harvesting**
  (fasting, cleaning)
- **Post-harvest processing**
  (freezing, blanching, drying, grinding, UV radiation, storage)

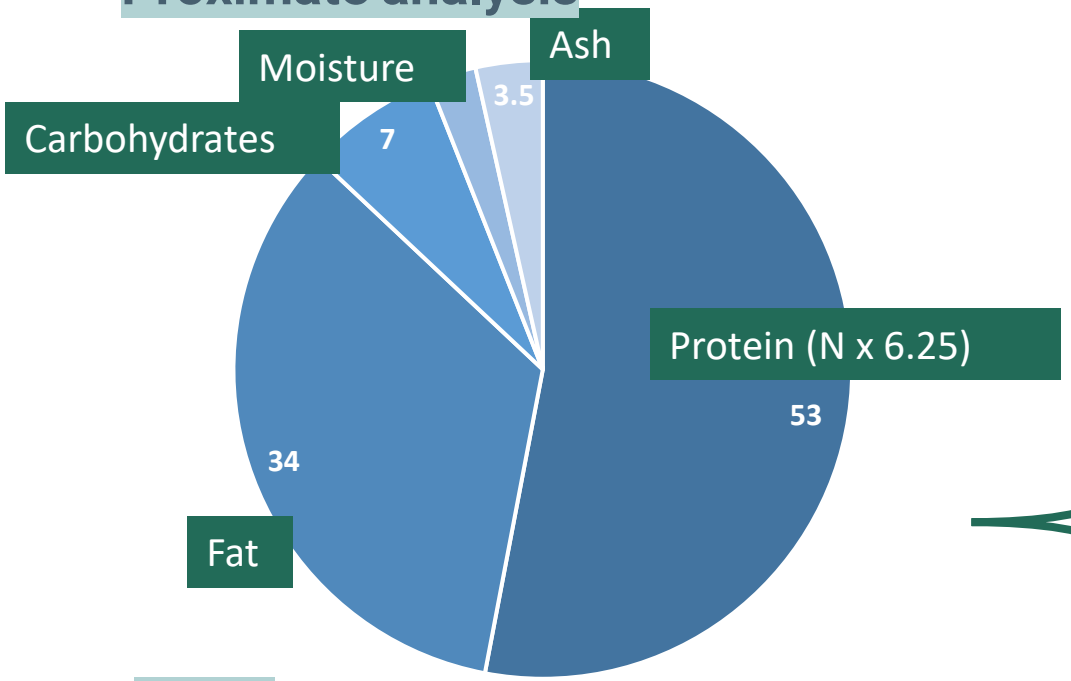
- Antibiotics, hormones
- Chemical substances
  (heavy metals, pesticide residues, PCBs, dioxins)
- Allergens in feed
- Biological hazards (bacteria, parasites, fungi, viruses)

CASE STUDY

Case Study - Compositional data

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

Proximate analysis



Chitin

- Main form of fibre in the NF
- Second most abundant biopolymer
- Challenges - analytical quantification

3.4. Compositional data

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided qualitative and quantitative data on chemical and microbiological parameters for a number of different batches of the NF. The Panel notes that not all the analyses have been performed on the same batches of the NF.

Certificates of accreditation for the laboratories that conducted the analyses were provided by the applicant. Analytical data were produced using methods validated for other types of matrices. Whenever in-house methods were employed, a full description of the method, as well as the results of the validation procedures, have been provided.

The NF mainly consists of crude protein, fat, and carbohydrates. The results of the proximate analysis of the NF are presented in Table 1. The amino acid, fatty acid, vitamin and mineral compositions are reported in Section '3.9 Nutritional information'.

Table 1: Batch-to-batch proximate analysis of the NF

Parameter (unit)	Batch number					Analytical method
	#1	#2	#3	#4	#5	
Crude protein (g/100 g)	53.1	52.5	52.3	53.6	53.7	Kjeldahl ($N \times 6.25$)
Crude fat (g/100 g)	34.1	33.9	35.3	33.9	33.9	Gravimetric method
Of which saturated (g/100 g)	7.61	7.60	7.90	7.34	7.28	Internal Method, GC/FID[a]
Total carbohydrates (g/100 g)	6.3	7.2	6.1	6.8	6.5	Calculation by difference[b]
Of which sugar (g/100 g)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	IC-PAD[c]
Ash (g/100 g)	3.5	3.5	3.4	3.8	3.9	Gravimetric method
Moisture (g/100 g)	3.0	2.9	2.9	1.9	2.0	Gravimetric method
Energy (kcal/100 g)	545	544	552	547	546	Regulation (EU) 1169/2011[d]
Energy (kJ/100 g)	2,272	2,270	2,300	2,281	2,278	Regulation (EU) 1169/2011[d]
	#6	#7	#8	#9	#10	
Dietary fibre (g/100 g)	4.2	4.1	3.6	3.3	3.5	Enzymatic – gravimetry

NF: novel food.
(a): GC-FID: gas chromatography with flame ionisation detection.
(b): Total carbohydrates = 100 – (crude protein + fat + ash + moisture).
(c): IC-PAD: ion chromatography-pulsed amperometric detection.
(d): Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304, 22.11.2011, p. 18-63.

Regarding the crude protein content of the NF, the Panel notes that Janssen et al. (2017b) suggest that it is possibly overestimated when using the nitrogen-to-protein conversion factor of 6.25, mainly due to the presence of chitin. This issue will be addressed in detail in Section '3.9 Nutritional information'.

Chitin is the main form of crude fibre in *T. molitor* larvae (Finke, 2007; Hahn et al., 2018; Han and Heinonen, 2020). It is a linear polysaccharide consisting of varying amounts of β-(1,4)-linked 2-amino-2-deoxy-β–glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose units (Muzzarelli and Raa, 1973; Roberts, 1992). After cellulose, chitin is the second most abundant natural biopolymer and occurs predominantly in the shells of crustaceans, the cell walls of fungi and the exoskeletons of insects (Muzzarelli et al., 1986; Dutta et al., 2004; Muthukrishnan et al., 2016). The physicochemical nature of chitin is intrinsically related to its source (Kumirska et al., 2011). The applicant provided analytical data on the levels of chitin in five independently produced batches of the NF. The Panel notes that a nationally or internationally recognised reference method for the analytical determination of chitin in insects does not exist. The chitin content in the NF was determined based on the protocol described by Hahn et al. (2018), in which chemical treatment [based on acid detergent fibre (ADF)-acid detergent lignin (ADL)] is used to estimate the chitin content. The Panel considers that the differences between the content of dietary fibre (Table 1) and chitin (Table 2) could be due to the different analytical methods utilised. Additionally, the Panel notes that the analytical results in Tables 1 and 2 do not concern the same NF batches.

Table 2: Chitin content in the NF, on a product basis

Parameters (g/100 g)	Batch number				
	#11	#12	#13	#14	#15
ADF[a]	7.7	9.6	7.5	7.9	7.1
ADL[b]	1.4	1.5	1.3	1.5	1.2
Chitin[c]	6.3	8.1	6.2	6.4	5.9

NF: novel food.
(a): ADF: acid detergent fibre.
(b): ADL: acid detergent lignin.
(c): Chitin calculated as ADF-ADL.

CASE STUDY

Case Study - Compositional data

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

Concentrations of heavy metals in the NF analysed by ICP-MS are reported in Table 3. The applicant compared the values to the maximum levels (MLs) for other foods as set in Regulation (EC) No 1881/2006[9]. The Panel notes that the concentrations of heavy metals reported for the NF do not exceed the maximum levels set for other foods and that they are similar to the concentrations previously reported and assessed for other foods derived from whole insects (EFSA NDA Panel, 2021a, b,c,d), and that in the current EU legislation, no maximum levels of heavy metals are set for insects and products thereof as food.

Table 3: Heavy metals in the NF

Heavy metals (mg/kg)	Batch number					Analytical method
	#16	#17	#18	#3	#19	
Lead	< 0.02	< 0.02	< 0.01	< 0.01	< 0.01	ICP-MS[(a)]
Cadmium	0.049	0.043	0.034	0.028	0.035	
Mercury	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	
	#20	#4	#21	#22	#23	
Arsenic	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	ICP-MS[(a)]

NF: novel food.
(a): ICP-MS: inductively coupled-plasma mass spectrometry.

Analytical data on the levels of aflatoxins B1, B2, G1, G2, ochratoxin A, deoxynivalenol, fumonisins B1 and B2, and zearalenone in the NF have been provided (Table 4). The values reported are below the limit of quantification (LOQ) of the analytical methods implemented. The LOQ values are lower than the MLs set for other foodstuffs in Regulation (EC) No 1881/2006. The Panel notes that in the current EU legislation no MLs of mycotoxins are set for insects as food.

Additionally, the concentrations of dioxins and dioxin-like PCBs in the NF were provided by the applicant (Table 5) and the values reported were lower than the MLs set for different foods in Regulation (EC) No 1881/2006, and comparable to those previously reported and assessed for other

Table 4: Mycotoxins in the NF, on a product basis

Parameter (µg/kg)	Batch number					Analytical method
	#24	#25	#18	#3	#26	
Aflatoxins B1	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	IAC-LC-FLD[(a)]
Aflatoxins B2	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	
Aflatoxins G1	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	
Aflatoxins G2	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	
Aflatoxins (Sum of B1, B2, G1, G2)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Ochratoxin A	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	
Deoxynivalenol	< 50	< 50	< 50	< 50	< 50	LC–MS/MS[(b)]
	#27	#28	#29	#30	#31	
Fumonisin B1	< 200	< 200	< 200	< 200	< 200	LC–MS/MS[(b)]
Fumonisin B2	< 200	< 200	< 200	< 200	< 200	
	#3	#4	#32	#18	#1	
Zearalenone	< 10	< 10	< 10	< 10	< 10	LC–MS/MS[(b)]

NF: novel food.
(a): IAC–LC-FLD: immunoaffinity chromatography–liquid chromatography/fluorescence detection.
(b): LC–MS/MS: liquid chromatography–tandem mass spectrometry.

foods derived from whole insects (EFSA NDA Panel, 2021a,b,c,d). The Panel notes that in the current EU legislation, no maximum levels of dioxins and dioxin-like compounds are set for insects and products thereof as food.

Analytical data on the pesticide residue levels on four independently produced batches of the NF have been provided. The results showed that all the analysed pesticides in the NF are below the limits of detection (LODs) or LOQs of the analytical multimethod used (ASU L00.00–34).

Table 5: Dioxins and dioxin-like PCBs in the NF

Dioxins (pg/g fat)	Batch number					Analytical method
	#32	#18	#1	#3	#4	
WHO (2005)[(a)] PCDD/F + PCB TEQ (upper-bound)	0.255	0.261	0.257	0.262	0.255	EC 2017/644, GC–MS/MS[(b)]

NF: novel food; WHO (2005) PCDD/F + PCB TEQ: sum of polychlorinated dibenzo-p-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent.
(a): Van den Berg et al. (2006).
(b): GC–MS/MS: gas chromatography–tandem mass spectrometry.

Chemical Contaminants

- Heavy metals (lead, cadmium, mercury, arsenic)
- Mycotoxins (aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, zearalenone)
- Dioxins and dioxin-like PCBs
- Processing contaminants

Table 8: Processing contaminants in the NF

Parameter (unit)	Batch number					Analytical method
	#43	#44	#45	#46	#47	
Acrylamide (µg/kg)	< 20	< 20	26	< 20	< 20	LC–MS/MS[(a)]
	#48	#23	#49	#50	#51	
Chloropropanol (2-MCPD) (µg/kg)	< 10	< 10	< 10	< 10	< 10	GC-MS/MS[(b)]
Chloropropanol (3-MCPD) (µg/kg)	< 10	< 10	< 10	< 10	< 10	

NF: novel food.
(a): LC-MS/MS: Liquid chromatography–tandem mass spectrometry.
(b): GC-MS/MS: Gas chromatography–tandem mass spectrometry.

CASE STUDY

Case Study - Compositional data

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

Table 6: Microbiological analyses of the NF

Parameter (unit)	Batch number					Analytical method
	#33	#34	#35	#36	#37	
Aerobic plate count (30°C) (CFU/g)	7×10^4	$< 4 \times 10^4$	6×10^4	1.9×10^4	2.4×10^4	NF EN ISO 4833-1 or XP V08-034[(a)]
Yeasts and moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	NF V 08–036
Sulfite-reducing anaerobes (CFU/g)	< 10	< 10	< 10	< 10	< 10	NF V 08–061
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	NF EN ISO 7937
Bacillus cereus (CFU/g)	< 100	< 100	< 100	< 100	< 100	NF EN ISO 7932
L. monocytogenes in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	AES 10/03–09/00
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	NF V 08–054
β-Glucuronidase-positive *Escherichia coli* (CFU/g)	< 10	< 10	< 10	< 10	< 10	NF ISO 16649-2
Salmonella in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	Qualitative Rapid Salmonella alternative analytical method (certified under BRD 07/11–12/05)
Coagulase-positive staphylococci (CFU/g)	< 100	< 100	< 100	< 100	< 100	NF EN ISO 6888-1

NF: novel food; CFU: colony forming unit; EN: Europaische Norm (European Standard).
(a): Method NF EN ISO 4833-1 refers to batches #33, #34, #35 and method XP V08-034 to batches #36, #37. XP V08-34 is a method derived from NF EN ISO 4833-1.

The applicant provided analytical data for biogenic amines (cadaverine, spermine, tyramine, tryptamine, 2-phenylethylamine, histamine, putrescine and spermidine) for five independently produced batches of the NF (Table 7). Additional analyses have been performed on NF batches at t = 6 months, and the results are further discussed under Section '3.4.2 Stability'.

No legal MLs have been established for spermidine and spermine in foods. Higher concentrations have been reported in legumes/soybean products (up to 207 mg/kg and up to 69 mg/kg, respectively) and cereals (up to 353 mg/kg and up to 146 mg/kg, respectively), while lower values have been reported in fresh meat (13 mg/kg and 69 mg/kg, respectively) and cheese (38 mg/kg and 3 mg/kg, respectively) (Muñoz-Esparza et al., 2019). The histamine values were much lower than the limit of 200 mg/kg for histamine in fishery products set in Regulation (EC) No 2073/2005[10]. The Panel notes the levels of putrescine reported in the NF and that no legal limit has been established for putrescine in any food, although it may accumulate at very high concentrations in cheese (up to 1,560 mg/kg), fermented sausages (up to 1550 mg/kg) and fish sauces (up to 1,220 mg/kg) (EFSA BIOHAZ Panel, 2011). Tyramine levels in NF are much lower than levels reported in other foods such as cheese (Andersen et al., 2019).

Table 7: Biogenic amines levels of the NF

Parameter (mg/kg)	Batch number					Analytical method
	#38	#39	#40	#41	#42	
Cadaverine	6.66	7.02	8.01	7.53	7.45	Czech J. Food Sci. Vol.21, LC-UV/DAD[(a)]
Spermidine	3.44	180	4.01	179	172	
Spermine	53.1	56.1	51.7	56.7	52.2	
Histamine	< 1	1.03	< 1	< 1	1.45	
Putrescine	532	522	519	531	514	
Tyramine	4.78	6.20	4.36	5.26	7.15	
Tryptamine	< 5	< 5	< 5	< 5	< 5	
2-Phenylethylamine	66.4	51.5	67.0	50.3	48.7	

Escherichia coli

Case Study - Compositional data

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

The effect of UV treatment

- UVB light on the powder
- Investigate the impact on the compositional profile of the NF
- Conversion rate (~0.8%)

- Photoisomers: lumisterol, tachysterol
- Method development & validation
- No accumulation of photoisomers in the NF

3.4.1. The effect of UV treatment

Upon EFSA's request, the applicant investigated further the effect of the UV treatment on the yellow mealworm powder, by providing analytical data on the composition of the insect powder before and after the UVB radiation (proximate analysis, vitamin D3 and 7-dehydrocholesterol, lumisterol 3 and tachysterol 3).

The detailed proximate analysis results on the insect powder, before and after the UV treatment, are presented in Appendix A. The Panel notes that the batches of the insect powder before and after UV treatment (NF) tested are not always the same. The Panel concludes that the insect powder before UV treatment does not differ substantially to the NF (insect powder after UV treatment) in terms of proximate parameters (Appendix A).

Table 9: Vitamin D3 and 7-dehydrocholesterol (precursor) levels of yellow mealworm powder before and after UV treatment

Parameter (unit)	Batch number										Analytical method
	*(#132)	*(#142)	*(#140)	*(#111)	*(#143)	#52	#53	#54	#3	#55	
	Before UV treatment					After UV treatment					
Vitamin D3 (Cholecalciferol) (µg/100 g)	1.25	3.11	1.26	1.25	2.44	57.7	61.2	50.2	51.6	62.5	EN 12821:2009, LC-DAD[(a)]
	*(#56)	*(#57)	*(#58)	*(#59)	*(#60)	#56a	#57	#58	#59	#60	
7-Dehydrocholesterol (mg/kg fat)	55	199	240	210	210	241	211	240	256	267	Folch method

NF: novel food.
(a): LC-DAD: Liquid chromatography with diode array detection.
*: These are not NF batches.

Based on the results in Table 9, the Panel notes that the mean conversion rate of 7-dehydrocholesterol to vitamin D3 upon UV treatment is low (~ 0.8%). Because of this conversion rate, the Panel requested the applicant to investigate the formation of vitamin D3 photoisomers, in order to clarify whether an accumulation of these compounds occurs. According to Wacker and Holick (2013), the levels of these photoisomers may increase under UV radiation over time. The applicant provided analytical data on the levels of vitamin D3 and its photoisomers lumisterol 3 and tachysterol 3, on five batches of yellow mealworm powder, before and after UV treatment (Table 10). Regarding the analyses on vitamin D3 and 7-dehydrocholesterol levels (Table 9), the Panel notes that the batches of the insect powder before and after UV treatment (NF) tested are not the same. To perform the analysis, the applicant developed an in-house analytical protocol. The extraction of vitamin D3 from the NF was based on the method developed by Temova and Roškar (2016), and the detection of the target molecules via reverse-phase high-performance liquid chromatography with diode array detection (HPLC-DAD) on the protocol of Wittig et al. (2013). A full description of the analytical protocol implemented, as well as data demonstrating the respective validation procedures for the quantification of vitamin D3 have been provided.

Table 10: Vitamin D3, lumisterol 3 and tachysterol 3 levels in yellow mealworm powder, before and after the UV treatment

Parameters (unit)	Before UV treatment					After UV treatment (NF)					Analytical method
	*(#61)	*(#62)	*(#63)	*(#64)	*#65	#61	#62	#63	#64	#65	
Vitamin D3 (µg/100 g)	< 10	< 10	< 10	< 10	< 10	61	62	61	59	61	EN 12821:2009, LC-DAD[(a)]
Tachysterol 3 (µg/100 g)	125	128	122	111	110	252	182	199	200	203	HPLC-DAD[(b)] (internal method)
Lumisterol 3 (µg/100 g)	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50	< 50	

NF: novel food.
< 10 µg/100 g, < 50 µg/100 g are the LOQs, for vitamin D3 and lumisterol 3, respectively.
(a): LC-DAD: liquid chromatography with diode array detection.
(b): HPLC-DAD: high-performance liquid chromatography with diode array detection.
*: These are not NF batches.

According to these results, the Panel concluded that there is no substantial accumulation of the vitamin D3 photoisomers, tachysterol 3 and lumisterol 3, despite the low conversion of 7- dehydrocholesterol to vitamin D3.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

CASE STUDY

Case Study - Compositional data

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

Stability of the NF

- Data up to 6 months
- Microbiological aspects
- Chemical aspects
- Oxidative status of fats
- Vitamin D3 status
- Biogenic amines
- Stability of the NF in the proposes-for-use matrices

3.4.2. Stability

The applicant performed stability tests with several independently produced batches of the NF. The NF is to be stored in hermetically closed opaque packaging, at room temperature (~ 50% relative humidity), with an intended shelf life of 6 months. The tests were carried out at normal storage conditions for a period of 6 months. The microbiological profile of the NF (Table 11), the oxidative status of fat (Table 12), water activity (Table 12), vitamin D3 levels (Table 13), as well as biogenic amines (Appendix B) were investigated. The Panel notes that the five batches of the NF analysed at t = 6 months are not the same five NF batches analysed at t = 0 months, with the exception of vitamin D3 (Table 13).

Table 11: Microbiological status of the NF during the proposed shelf life

Parameter	Batch number										Analytical method
	#33	#34	#35	#36	#37	#66	#67	#68	#69	#70	
Time (months)	0					6					
Aerobic plate count (30°C) (CFU/g)	7×10^4	$< 4 \times 10^6$	6×10^4	1.9×10^4	2.4×10^4	$< 10^3$	$< 10^3$	6×10^3	7×10^4	$< 4 \times 10^4$	NF EN ISO 4833-1 and XP V08-034[a]
Yeasts and moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	NF V 08-036
Sulfite-reducing anaerobes (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Internal, NF V 08-061
Clostridium perfringens (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Internal, NF EN ISO 7937
Bacillus cereus (CFU/g)	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	Internal, NF EN ISO 7932
L. monocytogenes in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	AES 10/03-09/00
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	NF V 08-054
β-Glucuronidase-positive *Escherichia coli* (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Internal, NF ISO 16649-2
Salmonella in 25 g	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Qualitative Rapid Salmonella alternative analytical method (certified under BRD 07/11-12/05)
Coagulase-positive staphylococci (CFU/g)	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	Internal, NF EN ISO 6888-1/A2 and NF V08-057-1[b]

CFU: colony forming unit; NF: novel food; N.D.: Not detected.
(a): Method NF EN ISO 4833-1 refers to batches #33, #34, #35, #68, #69, #70 and method XP V08-034 to batches #36, #37, N-02022 and #67.
(b): Method NF EN ISO 6888-1/A2 refers to batches #33, #34, #35, #36, #37, #68, #69 and #70 and method NF V08-057-1 refers to #66 and #67.

The Panel notes that the microbiological values do not exceed the given specification limits.

Table 12: Water activity and oxidative status of fat of the NF during the proposed shelf life

Parameter (unit)	Batch number										Analytical method
Time (months)	0					6					
	#71	#27	#5	#72	#73	#74	#24	#25	#75	#76	
p-anisidine value	0.7	0.6	< 0.5	0.9	0.9	< 0.5	0.6	0.5	< 0.5	< 0.5	Internal Spectrophotometry
	#71	#27	#5	#72	#73	#74	#24	#25	#75	#76	
Peroxide value (meq O_2/kg fat)	1.0	1.3	2.2	2.2	1.6	1.8	1.8	2.8	2.4	4.0	Internal, Titrimetry
	#55	#32	#77	#18	#3	#78	#79	#80	#81	#82	
Acid value (mg KOH/g)	3.0	3.1	3.0	3.0	3.0	3.0	2.9	2.8	2.8	2.8	Internal, Titrimetry
FFA (% in oil)	1.52	1.56	1.49	1.49	1.52	1.48	1.48	1.42	1.43	1.42	
	#83	#84	#85#85	#86	#87	#72	#88	#89	#90	#91	
a_w	0.157	0.156	0.158	0.164	0.162	0.164	0.159	0.168	0.166	0.171	Internal, Hygrometry (dew point)

FFA: Free fatty acids; NF: novel food.

The applicant provided analytical data on the water activity and on the oxidative status of five independently produced batches for time 0 and 6 months, measuring the *p*-anisidine value, peroxide value, acid value and % FFA in fat. The Panel notes that the values do not exceed the respective specification limits.

Table 13: Vitamin D3 contents of the NF during the proposed shelf life

Parameter (unit)	Time (months)	Batch number					Analytical method
		#56a	#56b	#58	#59	#60	
Vitamin D3 (µg/100 g)	0	45.6 ± 11.9	49.4 ± 12.8	44.1 ± 11.5	54.4 ± 14.1	52.3 ± 13.6	EN 12821:2009, LC-DAD[(a)]
	6	53.4 ± 13.9	51.1 ± 13.3	48.9 ± 12.7	47.1 ± 12.2	59.8 ± 15.6	

NF: novel food.
(a): LC-DAD: Liquid chromatography with photodiode array detection.

The contents of vitamin D3 were examined at t = 0 and at the end of the proposed shelf life (6 months). The stability test indicated that there were no substantial changes in vitamin D3 content.

The applicant provided analytical data for biogenic amines for five different batches at t = 6 months (Appendix B). Also considering the data in Table 7 at t = 0, the Panel concludes that there is no evidence for the accumulation of biogenic amines in the NF during storage. Upon EFSA's request, the applicant analysed the NF for *Pseudomonas aeruginosa*, which could have contributed to the occurrence of biogenic amines in the NF. However, this seems not to be the case since *P. aeruginosa* was reported at levels < 1 CFU/g (method: adapted from NF EN ISO 16266).

The Panel considers that the data provided sufficient information with respect to the stability of the NF with a shelf life of 6 months.

Stability in the intended-for-use food matrices

Since the NF is going to be used as an ingredient of other food products, EFSA asked the applicant to investigate the stability when the NF is used as an ingredient in the intended-for-use matrices (see Section 3.7.2 Proposed uses and use levels).

The applicant investigated the forming of processing contaminants, i.e. acrylamide (LC-MS/MS), 2-MCPD and 3-MCPD (GC-MS/MS) in cakes prepared with and without the NF as an ingredient (1 sample per cake). The recipe was modified by replacing part of the wheat flour and oil with the NF in a way that resulted in the same fat concentrations in the two products. The Panel notes that the acrylamide concentration in the cake containing the NF did not increase, compared to the cake without the NF. The concentrations of 2- and 3-MCPD were below the LOQ (10 µg/kg) of the analytical method implemented, in both preparations.

Moreover, the applicant provided data on the microbiological profile of a fruit puree with the NF as an ingredient during its shelf life (6 months). The Panel notes that the resulting microbiological values did not raise any safety concerns.

The Panel further notes that the food items containing the NF have to comply with currently established legislative limits, such as microbiological levels set in Regulation (EC) 2073/2005 and the benchmark levels of acrylamide in bakery products established by Regulation (EU) No 2017/2158[11]. The stability data on microbial contamination in the fruit puree matrix tested did not raise safety concerns at the end of the shelf life.

Provided that the NF specifications are met at the end of the shelf life, and that products containing the NF as an ingredient are compliant with respective legislative limits on processing contaminants, the stability data do not raise safety concerns.

CASE STUDY

Case Study - Specifications

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

Table 14: Specifications of the NF

Description: whole, blanched, oven-dried, ground and UVB-radiated *Tenebrio molitor* larvae (powder)		
Parameter	**Unit**	**NF**
Appearance	–	Dark brown powder
a_w	–	< 0.6
Peroxide value	meq O_2/kg fat	≤ 5
***p*-anisidine value**	–	≤ 1
Moisture	% w/w	1.4–3.5
Ash	% w/w	3–4
Crude protein	% w/w	50–55
Total carbohydrates	% w/w	6–7.5
Dietary fibre	% w/w	3–4.5
Chitin	% w/w	5.5–8.5
Fat	% w/w	30–37
Vitamin D3	µg/100 g	35–79
Copper	mg/kg	13–16
Manganese	mg/kg	9–11.5
Lead	mg/kg	≤ 0.02
Cadmium	mg/kg	≤ 0.1
Mercury	mg/kg	≤ 0.005
Arsenic	mg/kg	≤ 0.05
Microbiological		
Bacillus cereus	CFU/g	≤ 100
Clostridium perfringens	CFU/g	≤ 10
β-Glucuronidase-positive *Escherichia coli*	CFU/g	≤ 10
Aerobic mesophilic bacteria	CFU/g	≤ 10^5
Listeria monocytogenes	In 25 g	Not detected
Yeasts and moulds	CFU/g	≤ 10
Enterobacteriaceae	CFU/g	≤ 10
Coagulase-positive staphylococci	CFU/g	≤ 100
Sulfite-reducing anaerobes	CFU/g	≤ 10
***Salmonella* spp.**	in 25 g	Not detected
Mycotoxins		
Aflatoxin B1	µg/kg	≤ 0.1
Aflatoxin B2	µg/kg	≤ 0.1
Aflatoxin G1	µg/kg	≤0.1
Aflatoxin G2	µg/kg	≤ 0.2
Aflatoxin (Sum of B1 + B2, G1 + G2)	µg/kg	≤ 0.5
Fumonisin B1 + B2	µg/kg	≤ 400
Ochratoxin A	µg/kg	≤ 0.2
Deoxynivalenol	µg/kg	≤ 50
Zearalenone	µg/kg	≤ 10

NF: novel food; w/w: weight per weight; CFU: colony forming unit.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

- Key parameters for characterisation and identity
- Rationale for the selected parameters provided
- Ranges/limits regarding identity
- Limits for substances of concern
- Proposed by applicant
- EFSA can amend them
- Used by legislators for the marketing authorization
- Can be amended by legislators
- Serve for market control purposes

CASE STUDY

Case Study - Proposed uses

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

As the NF is intended to be used as an ingredient in standard food categories, the NF can be consumed by any group of the population. Therefore, the target population is the general population, and the safety data and the exposure assessment shall cover all population groups (Commission Implementing Regulation (EU) 2017/2469, Article 5(6)).

3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in several food products. The food categories defined using the FoodEx2 hierarchy (EFSA, 2015) and the maximum use levels are reported in Table 15.

Table 15: Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)
4	A004X	Wheat bread and rolls	4
3	A00AN	Cakes	4
3	A007D	Pasta and similar products	3.5
4	A01PD	Compote of fruit/vegetables	3.5
3	A0DPP	Potatoes and similar	3
2	A02QE	Cheese	1

NF: novel food.

3.7.3. Anticipated intake of the NF

EFSA assessed the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 15), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intake of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 16.

Table 16: Intake estimate of the NF resulting from its use as an ingredient in the intended food categories at the maximum proposed use levels

Population group	Age (years)	Mean intake (mg/kg bw per day)		P95 intake (mg/kg bw per day)	
		Lowest[a]	Highest[a]	Lowest[b]	Highest[b]
Infants	< 1	24	309	227	845
Young children[d]	1 to < 3	178	404	362	773
Other children	3 to < 10	112	388	280	744
Adolescents	10 to < 18	63	181	122	393
Adults[c]	≥ 18	47	143	109	303

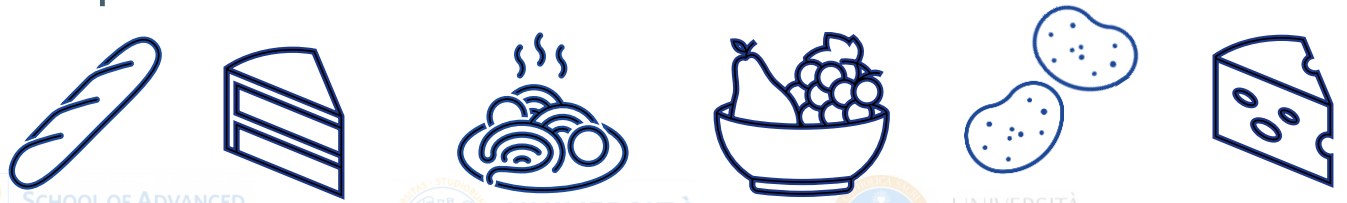
NF: novel food; bw: body weight.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 28/2/2023. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 28/2/2023. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 based on less than 60 individuals are not considered).
(c): Includes elderly, very elderly, pregnant and lactating women.
(d): Referred to as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

The estimated daily intake of the NF for each population group from each EU dietary survey is available in the Excel file annexed to this scientific opinion (under supporting information).

3.7.4. Estimate of exposure to undesirable substances

Based on the highest P95 intake estimate (Table 16), EFSA estimated exposure to undesirable substances (heavy metals, toxins) from the NF, for all population groups. The specification limits (Table 14) were used as the maximum concentrations of the undesirable substances. When specification limits for a substance of possible concern have not been proposed, the maximum values reported for the analysed batches were used. The Panel considers that consumption of the NF under the proposed uses and use levels does not contribute substantially to the overall dietary intake of the analysed undesirable substances. The assessment of the intake of manganese (Mn) from the NF is provided in Section '3.9 Nutritional information'.

- Target population: general
- Use: food ingredient
- Food categories: wheat bread and rolls, cakes, pasta, compote of fruits/vegetables, potatoes and similar, cheese
- Exposure: undesirable substances and nutrients

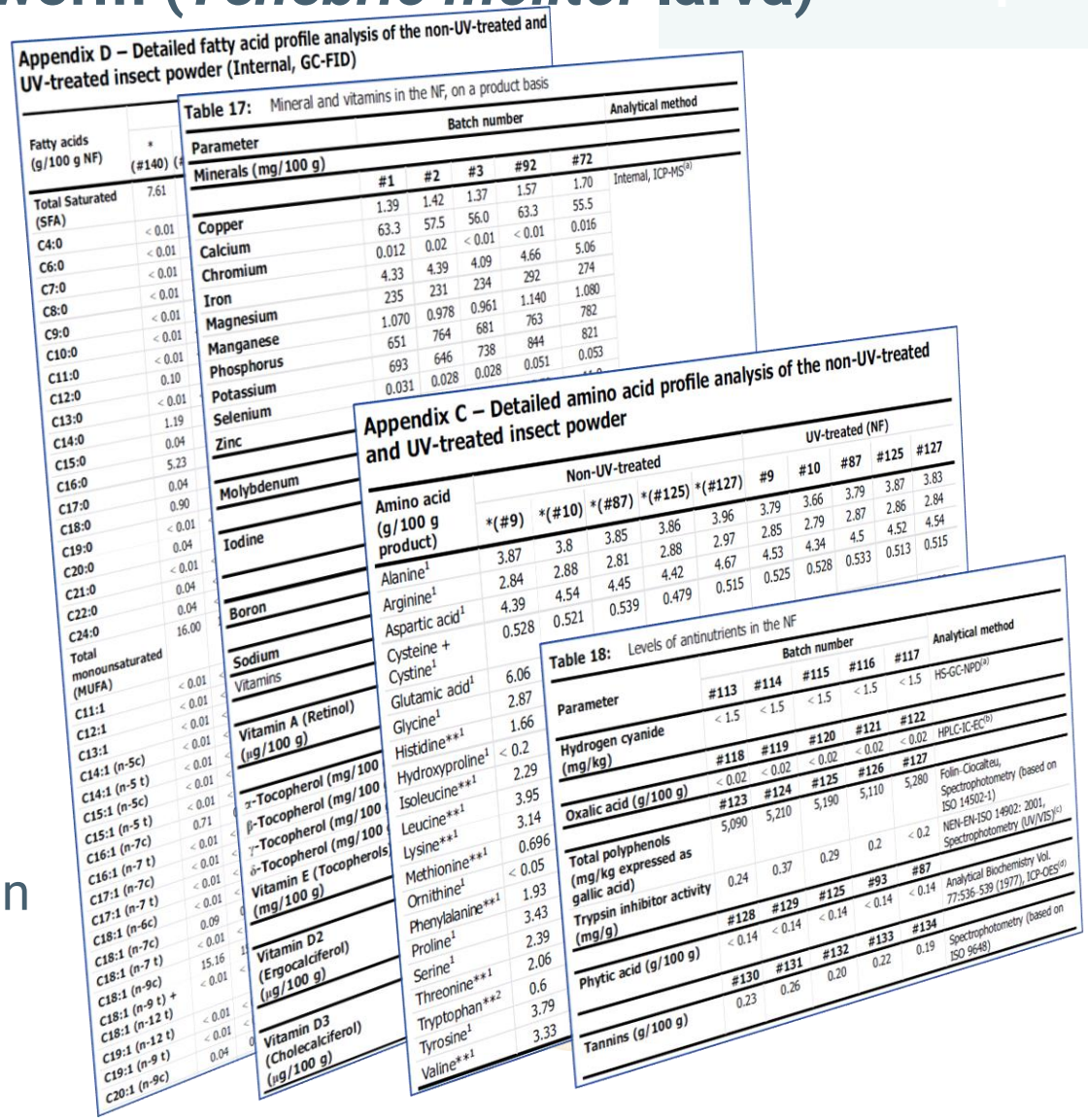


CASE STUDY

Case Study - Nutrition

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

- Energy value
- Protein quantity & quality
- Amino acid profile
- Fatty acid profile
  (saturated = ~23.1%, monounsaturated: ~53%, polyunsaturated= ~23.5%
- Vitamins
- Minerals
- Vitamin D3: not a significant dietary contributor
- Chitin
- Antinutritional factors (e.g., oxalic acid, hydrogen cyanide, trypsin inhibitors)



Appendix D – Detailed fatty acid profile analysis of the non-UV-treated and UV-treated insect powder (Internal, GC-FID)

Fatty acids (g/100 g NF)	* (#140)
Total Saturated (SFA)	7.61
C4:0	< 0.01
C6:0	< 0.01
C7:0	< 0.01
C8:0	< 0.01
C9:0	< 0.01
C10:0	< 0.01
C11:0	< 0.01
C12:0	0.10
C13:0	< 0.01
C14:0	1.19
C15:0	0.04
C16:0	5.23
C17:0	0.04
C18:0	0.90
C19:0	< 0.01
C20:0	0.04
C21:0	< 0.01
C22:0	0.04
C24:0	0.04
Total monounsaturated (MUFA)	16.00
C11:1	< 0.01
C12:1	< 0.01
C13:1	< 0.01
C14:1 (n-5c)	< 0.01
C14:1 (n-5 t)	< 0.01
C15:1 (n-5c)	< 0.01
C15:1 (n-5 t)	0.71
C16:1 (n-7c)	< 0.01
C16:1 (n-7 t)	< 0.01
C17:1 (n-7c)	< 0.01
C17:1 (n-7 t)	< 0.01
C18:1 (n-6c)	0.09
C18:1 (n-7c)	< 0.01
C18:1 (n-7 t)	15.16
C18:1 (n-9c)	< 0.01
C18:1 (n-9 t) + C18:1 (n-12 t)	< 0.01
C19:1 (n-12 t)	< 0.01
C19:1 (n-9 t)	0.04
C20:1 (n-9c)	

Table 17: Mineral and vitamins in the NF, on a product basis

Parameter	Batch number #1	#2	#3	#92	#72	Analytical method
Minerals (mg/100 g)						
Copper	1.39	1.42	1.37	1.57	1.70	Internal, ICP-MS[a]
Calcium	63.3	57.5	56.0	63.3	55.5	
Chromium	0.012	0.02	< 0.01	< 0.01	0.016	
Iron	4.33	4.39	4.09	4.66	5.06	
Magnesium	235	231	234	292	274	
Manganese	1.070	0.978	0.961	1.140	1.080	
Phosphorus	651	764	681	763	782	
Potassium	693	646	738	844	821	
Selenium	0.031	0.028	0.028	0.051	0.053	
Zinc						
Molybdenum						
Iodine						
Boron						
Sodium						
Vitamins						
Vitamin A (Retinol) (µg/100 g)						
α-Tocopherol (mg/100						
β-Tocopherol (mg/100						
γ-Tocopherol (mg/100						
δ-Tocopherol (mg/100						
Vitamin E (Tocopherols (mg/100 g)						
Vitamin D2 (Ergocalciferol) (µg/100 g)						
Vitamin D3 (Cholecalciferol) (µg/100 g)						

Appendix C – Detailed amino acid profile analysis of the non-UV-treated and UV-treated insect powder

Amino acid (g/100 g product)	Non-UV-treated *(#9)	*(#10)	*(#87)	*(#125)	*(#127)	UV-treated (NF) #9	#10	#87	#125	#127
Alanine[1]	3.87	3.8	3.85	3.86	3.96	3.79	3.66	3.79	3.87	3.83
Arginine[1]	2.84	2.88	2.81	2.88	2.97	2.85	2.79	2.87	2.86	2.84
Aspartic acid[1]	4.39	4.54	4.45	4.42	4.67	4.53	4.34	4.5	4.52	4.54
Cysteine + Cystine[1]	0.528	0.521	0.539	0.479	0.515	0.525	0.528	0.533	0.513	0.515
Glutamic acid[1]	6.06									
Glycine[1]	2.87									
Histidine**[1]	1.66									
Hydroxyproline[1]	< 0.2									
Isoleucine**[1]	2.29									
Leucine**[1]	3.95									
Lysine**[1]	3.14									
Methionine**[1]	0.696									
Ornithine[1]	< 0.05									
Phenylalanine**[1]	1.93									
Proline[1]	3.43									
Serine[1]	2.39									
Threonine**[1]	2.06									
Tryptophan**[2]	0.6									
Tyrosine[1]	3.79									
Valine**[1]	3.33									

Table 18: Levels of antinutrients in the NF

Parameter	Batch number #113	#114	#115	#116	#117	Analytical method
Hydrogen cyanide (mg/kg)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	HS-GC-NPD[a]
	#118	#119	#120	#121	#122	
Oxalic acid (g/100 g)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	HPLC-IC-EC[b]
	#123	#124	#125	#126	#127	
Total polyphenols (mg/kg expressed as gallic acid)	5,090	5,210	5,190	5,110	5,280	Folin-Ciocalteu, Spectrophotometry (based on ISO 14502-1)
Trypsin inhibitor activity (mg/g)	0.24	0.37	0.29	0.2	< 0.2	NEN-EN-ISO 14902: 2001, Spectrophotometry (UV/VIS)[c]
	#128	#129	#125	#93	#87	
Phytic acid (g/100 g)	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	Analytical Biochemistry Vol. 77:536-539 (1977), ICP-OES[d]
	#130	#131	#132	#133	#134	
Tannins (g/100 g)	0.23	0.26	0.20	0.22	0.19	Spectrophotometry (based on ISO 9648)

CASE STUDY

Case Study – Toxicology & Allergenicity

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

3.10. Toxicological information

The Panel notes that no toxicological studies with the NFs were provided. The toxicological profile of *T. molitor* larvae has been previously assessed by the Panel (EFSA NDA Panel, 2021a,b). The Panel noted that *T. molitor* larvae should be reared separately from the adults since it has been reported that *T. molitor* adults may excrete potentially toxic substances as part of their defence mechanisms (Ladisch et al., 1967; Attygalle et al., 1991; Brown et al., 1992). The Panel also assessed toxicological studies available in the literature (*in vitro* and *in vivo* genotoxicity, acute, subacute and subchronic toxicity) with processed (freeze-dried) *T. molitor* larvae as the testing material (Han et al., 2014, 2016). The Panel concludes that the material assessed in these studies can be considered representative of the NF only with regards to the profile of the endogenously produced compounds of possible concern but not for any compounds that can be present due to the rearing conditions (e.g. feed) or processing (EFSA NDA Panel, 2021a,b).

Potential adverse health effects of chitin may be related to immunological effects. As reviewed by Komi et al. (2018), chitin has been shown to activate a variety of innate (eosinophils, macrophages) and adaptive immune cells (IL-4/IL-13 expressing T helper type-2 lymphocytes).

Taking into account the production process and the nature of the NF the Panel considers that no additional toxicological studies are required on the NF.

3.10.1. Human data

The applicant did not provide any human studies conducted with the NF or its source. No human studies were retrieved from the literature search.

3.11. Allergenicity

The Panel has previously considered that the consumption of the NF source (yellow mealworm), may trigger primary sensitisation to yellow mealworm proteins. The Panel has also considered that allergic reactions may occur in subjects allergic to crustaceans and dust mites due to cross-reactivity. Furthermore, the Panel has noted that additional allergens may end up in the NF if these allergens are present in the substrate fed to the insects (e.g. gluten). This may include allergens listed in the Annex II of Regulation (EU) No 1169/2011 (EFSA NDA Panel, 2021a,b).

The applicant provided data on the gluten content for five independently produced batches, analysed using Enzyme Linked Immunosorbent Assay (ELISA). The values were below 20 mg/kg. According to Commission Implementing Regulation (EU) No 828/2014[15], foods with gluten levels below 20 mg/kg are considered to be safe for consumption by individuals with celiac disease.

The Panel considers that the allergenicity risk is not expected to be greater compared to that associated with the consumption of non-UV-treated dried yellow mealworm. The additional UV treatment is not expected to alter the allergenicity risk.

Toxicological assessment

- Literature data
- Defence mechanism excretions
- No additional studies requested
- No human studies provided/requested

Allergenicity assessment

- Primary sensitisation
- Cross-allergenicity
- Allergens from the feed: gluten below 20mg/kg
- UV-treatment does not affect the allergenicity risk

CASE STUDY

Case Study - Discussion & Conclusion

UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)

Main Points

- Production process: sufficiently described, no concerns
- Contaminants mainly depend on their occurrence in the feed
- Stability: no concerns if specifications are met
- No substantial dietary contributor to undesirable substances
- Overestimation of true protein, but still high levels
- Chitin may affect bioavailability of minerals like other fibres
- The NF is not nutritionally disadvantageous
- No toxicological concerns
- Allergenic potential

Conclusion

The NF is safe under the proposed conditions of use

Recommendations

Research should be undertaken regarding the allergenicity of yellow mealworm, including cross-reactivity to other allergens

Proprietary Data

EFSA could not have reach the conclusion without certain data provided by the applicant and claimed as confidential

CASE STUDY

Q&A time

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Break

10:00

Start | Stop | Reset | mins: 10 | secs: 0 | type: None

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