SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 28 March 2023

doi: 10.2903/j.efsa.2023.8009

Safety of UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA Panel), Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Margarita Aguilera-Gómez, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Miguel Prieto Maradona, Alexandros Siskos, Josef Rudolf Schlatter, Henk van Loveren, Panagiota Zakidou, Ermolaos Ververis and Helle Katrine Knutsen

Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The term yellow mealworm refers to the larval form of the insect species *T. molitor*. The NF is the UV-treated powder of the whole, thermally dried yellow mealworm. The NF consists mainly of crude protein, fat, digestible carbohydrates and fibre (chitin). The Panel notes that the levels of contaminants in the NF highly depend on the occurrence levels of these substances in the insect feed. The Panel notes furthermore that there are no safety concerns regarding the stability of the NF if the NF complies with the proposed specification limits during its entire shelf life. The NF has a high protein content, although the true protein content in the NF is overestimated when using the nitrogen-to-protein conversion factor of 6.25, due to the presence of non-protein nitrogen. The applicant proposed to use the NF as an ingredient in various food products, such as bakery products, pasta, compotes of fruit/vegetables and cheese. The target population is the general population. The Panel notes that considering the composition of the NF, the proposed conditions of use and that the NF will not be the sole source of dietary protein, the consumption of the NF is not nutritionally disadvantageous. Despite the UV treatment, the Panel notes that the NF is not a significant dietary contributor of vitamin D3. The submitted toxicity studies from the literature did not raise safety concerns. The Panel considers that the consumption of the NF may induce primary sensitisation and allergic reactions to yellow mealworm proteins and may cause allergic reactions in subjects with allergies to crustaceans and dust mites. Additionally, allergens from the feed may end up in the NF. With the exception of possible allergenicity, the Panel concludes that the NF is safe under the proposed uses and use levels.

Keywords: novel foods, food safety, *Tenebrio molitor* larva, yellow mealworm, insect powder, UV radiation, edible insect

Requestor: European Commission

Question number(s): EFSA-Q-2019-00748

Correspondence: nif@efsa.europa.eu

Panel members: Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank Vânia Mendes who, as a trainee, provided support to the preparation of this scientific output.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Aguilera-Gómez M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Siskos A, Schlatter JR, van Loveren H, Zakidou P, Ververis E and Knutsen HK, 2023. Scientific Opinion on the safety of UV-treated powder of whole yellow mealworm (*Tenebrio molitor larva*) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2023;21(5):8009, 32 pp. https://doi.org/10.2903/j.efsa.2023.8009

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 16 March 2019, the company Nutri'Earth, submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place on the EU market *Tenebrio molitor* (mealworm) flour.

The *Tenebrio molitor* (mealworm) flour is intended to be used in a number of food applications. The applicant has requested data protection according to the provisions of Article 26 of Regulation (EU) 2015/2283.

On 15 June 2020, in accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on *Tenebrio molitor* (mealworm) flour.

During the course of the evaluation of this application, it became apparent that it is appropriate to update the request of the Commission to EFSA to more accurately describe the identity of the novel food, so as to reflect the use of ultraviolet light to increase the levels of vitamin D3 in the final food powder. On that basis, the Commission amended the title to "Updated request for a scientific opinion on UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva)".

In this opinion on UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva), the European Food Safety Authority should also document whether and to what extent the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283 are fulfilled regarding the data for which the applicant is requesting data protection.

1.2. Interpretation of the Terms of Reference

Given the proposed intended uses and in accordance with Article 5 of Commission Implementing Regulation (EU) 2017/2469[2] stating 'where it cannot be excluded that a novel food intended for a particular group of the population would be also consumed by other groups of the population, the safety data provided shall also cover those groups', it was clarified that the target population is the general population.

The applicant was requested to provide a revised assessment for the anticipated intake considering all population groups.

1.3. Additional information

On 24 November 2020, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) adopted a scientific opinion on the safety of dried yellow mealworm (*Tenebrio molitor* larva) as NF pursuant to Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels (EFSA NDA Panel, 2021a). Following a positive vote of the Standing Committee on Plants, Animals, Food and Feed (Novel Food and Toxicological Safety section) on 3 May 2021, the European Commission adopted on 1 June 2021 Commission Implementing Regulation (EU) 2021/882[3] authorising the placing on the market of dried yellow mealworm as an NF according to Regulation (EU) 2015/2283.

On 7 July 2021, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) adopted a scientific opinion on the safety of frozen and dried formulations from whole yellow mealworm (*T. molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels (EFSA NDA Panel, 2021b). Following a positive vote of the Standing Committee on Plants, Animals, Food and Feed (Novel Food and Toxicological Safety section) on 30 November 2021, the European Commission

[1] 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 1852/200. OJ L 327, 11.12.2015, p. 1–22.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

[3] Commission Implementing Regulation (EU) 2021/882 of 1 June 2021 authorising the placing on the market of dried *Tenebrio molitor* larva as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. OJ L 194, 2.6.2021, p. 16–20.

adopted on 8 February 2022 Commission Implementing Regulation (EU) 2022/169[4] authorising the placing on the market of frozen, dried and powder forms of yellow mealworm (*T. molitor* larva) as an NF according to Regulation (EU) 2015/2283.

2. Data and Methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information. During the assessment, the Panel identified additional data which were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469.

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise the detailed description of the production process, compositional analyses, specifications, a protein digestibility study and calculations on the Protein Digestibility Corrected Amino Acid Score (PDCAAS).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only the risks that might be associated with the consumption of the NF under the proposed conditions of use and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF subject of the application is the UV-treated powder of dried whole *T. molitor* larva (yellow mealworm), an insect species that belongs to the family of Tenebrionidae (darkling beetles). The NF falls under the category 'food consisting of, isolated from, or produced from animals or their parts', as described in Article 3(2)(v) of Regulation (EU) 2015/2283. The NF is produced by farming and processing yellow mealworms and consists mainly of crude protein, fat and carbohydrates. The NF is proposed to be used, in the form of a powder, as an ingredient in various food products such as bakery products, pasta, compotes of fruit/vegetables, and cheese. The NF will be added to foods intended for the general population.

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

[4] Commission Implementing Regulation (EU) 2022/169 of 8 February 2022 authorising the placing on the market of frozen, dried and powder forms of yellow mealworm (Tenebrio molitor larva) as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. OJ L 28, 9.2.2022, p. 10–16.

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.

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/

[5] Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. OJ L 189, 20.7.2007, p. 1–23.

[6] Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. OJ L 250, 18.9.2008, p. 1–84.

[7] Converted from International Units (IU) using the conversion factor of 0.025 μg =1 IU stated in the European Food Safety Authority Technical Report on Dietary Reference Values for nutrients (EFSA, 2017).

[8] Commission Implementing Regulation (EU) 2017/1492 of 21 August 2017 concerning the authorisation of cholecalciferol as a feed additive for all animal species. OJ L 216, 22.8.2017, pp. 19–22.

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.

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.

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

[9] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

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.

Given the vegetable origin of the feeding substrate and the absence of prion or prion-related encoding genes in insects, the development of specific prion diseases due to the consumption of the NF is not expected (EFSA Scientific Committee, 2015).

The applicant provided microbiological data on five independently produced batches of the NF (Table 6).

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

Parameter (unit)	Batch number					Analytical method
	#33	#34	#35	#36	#37	
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	

NF: novel food.
(a): LC-UV/DAD: Liquid chromatography/ultraviolet detection/diode array detection.

[10] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

Tryptamine was not quantified in any of the batches tested. Formation of biogenic amines can occur by endogenous biosynthesis, uptake from the feed source and by bacteria of the intestinal microbiota of insects. It can also occur during food processing and storage as a result of bacterial contamination (EFSA BIOHAZ Panel, 2011).

Regarding processing contaminants, the applicant provided data on acrylamide and chloropropanols (2- and 3-MCPD). Values were < LOQ, with the exception of batch #45 (acrylamide = 26 μg/kg). The Panel considers that the concentrations of the analysed processing contaminants do not raise safety concerns (Table 8).

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.

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.

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	$< 4 \times 10^4$	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

Parameter	Batch number										Analytical method
	#33	#34	#35	#36	#37	#66	#67	#68	#69	#70	
Time (months)	0					6					
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

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.

value, acid value and % FFA in fat. The Panel notes that the values do not exceed the respective specification limits.

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.

3.5. Specifications

The specifications of the NF are indicated in Table 14.

[11] Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. OJ L 304, 21.11.2017, pp. 24–44.

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.

3.6. History of use of the NF and/or of its source

There is no history of use of the NF. The source of the NF is the yellow mealworm (*T. molitor* larva). Yellow mealworms are consumed as part of the customary diet or for medicinal purposes in some non-EU countries (Thailand, China and Mexico) (Ramos-Elorduy, 1997; Ramos-Elorduy and Moreno, 2004; Ramos-Elorduy, 2009; Hanboonsong et al., 2013; Feng et al., 2018). Since 1 June

2021, certain food products from the same source (yellow mealworm) are authorised in the EU market for human consumption (Commission Implementing Regulation (EU) 2021/88)[12].

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).

[12] Commission Implementing Regulation (EU) 2021/882 of 1 June 2021 authorising the placing on the market of dried Tenebrio molitor larva as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017/2470. OJ L 194/16, 2.6.2021.

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'.

3.8. Absorption, distribution, metabolism and excretion (ADME)

No ADME data have been provided for the NF.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF which consists mainly of protein, fat and carbohydrates. The energy value of this NF is 2,280 kJ (547 kcal)/100 g of NF. Analytical data on the amino acid composition, the fatty acid content, antinutritional factors, minerals and vitamins have been provided for several batches of the NF.

3.9.1. Protein content and protein quality

The NF contains on average 53.0 ($\pm$ 0.6) g crude protein per 100 g calculated using a nitrogen-to-protein conversion factor of 6.25. The Panel notes that the use of this conventional factor overestimates the true protein content in the yellow mealworm due to the presence of non-protein nitrogen derived mainly from chitin (Janssen et al., 2017b). Based on the amino acid profile of the insects, Janssen et al. (2017b) proposed a conversion factor of 4.76 for yellow mealworm. Using this factor, the protein content of the NF amounts to 40.4 g/100 g. The applicant has also calculated a nitrogen-to-protein conversion factor tailored to the NF, using the methodological approach of Janssen et al. (2017b) and the data for the amino acid composition of the NF, to reflect the amount of true protein. Based on this factor (5.13), the protein content of the NF is on average 43.6 g/100 g. For regulatory purposes for nutritional labelling, protein is defined as the total nitrogen measured by the Kjeldahl method multiplied by a nitrogen-to-protein conversion factor of 6.25 (Regulation (EU) No 1169/2011 on the provision of food information to consumers).

Previously the true ileal protein digestibility of dried yellow mealworm (non-UV-treated) was found to be 64.0 $\pm$ 0.0% using a dynamic *in vitro* gastrointestinal model (EFSA NDA Panel, 2021b), and the 'Digestible Indispensable Amino Acid Score' (DIAAS) score corresponded to 51%, in line with the results reported in the literature. However, it was highlighted that results reported in the literature for the protein digestibility of dried yellow mealworm differ among the studies (due to differences in the processing, and/or different models used to investigate digestibility) (EFSA NDA Panel, 2021a).

In addition, the applicant attempted to determine the protein digestibility in one sample using an *in vitro* enzyme digestion method (Megazyme Protein Digestibility Assay Kit (K-PDCAAS)). The digestibility factor obtained was 0.98. From the information provided on the protocol it could not be concluded whether the results refer to the crude protein (*N*-content $\times$ 6.25) or to the true protein. Additionally, the Panel notes that data on the validation of the protein digestibility method used were not provided. Thus, the produced value was not further considered for the calculation of the 'DIAAS'.

The applicant quantified the amino acids in five batches of the NF according to ISO 13903:2005 and Commission Regulation (EC) No 152/2009[13] (Appendix C) and all indispensable amino acids (IAA) were found to be present.

To investigate further the effect of the UV treatment, the applicant quantified the amino acids in five batches of non-UV-treated insect powder (Appendix C). A paired sample student t-test performed

[13] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. OJ L 54, 26.2.2009, p. 1–130.

by the applicant showed that the amino acid values do not significantly differ between UV-treated and non-UV-treated batches. In the case of tryptophan, one unexplained high value was obtained in one out of five batches of the non-UV-treated yellow mealworm powder, and not considered in the statistical analyses. The Panel notes that UV treatment did not significantly impact the amino acids of the NF, when compared to non-UV-treated dried yellow mealworm.

Based on the recommended amino acid scoring patterns (FAO, 2013) using the protein conversion factor of 6.25 for calculation of the amounts of the individual amino acids in the protein, the first limiting amino acids in the NF were the sulphur containing ones (methionine and cysteine) for children (6 months to 3 years) and for other children (≥ 3 years), adolescents, and adults, while for infants (birth to 6 months) the limiting amino acid was tryptophane.

Based on the high (95th percentile) intake levels of the NF (Section 3.7.3, Table 16) with a maximum content of crude protein of 55% (Section 3.5, Table 14), the corresponding protein intake per kg bw per day from the NF would amount to 0.47 g for infants, 0.43 g for young children, 0.41 g for other children, 0.22 g for adolescents and 0.17 g for adults. Such intakes would correspond to less than half of dietary reference values (PRIs - population reference intakes) (EFSA NDA Panel, 2012) for protein for the respective age groups. The Panel considers that the consumption of the NF is not expected to negatively impact protein nutrition.

3.9.2. Fatty acids, vitamins, and minerals

The major fatty acids in the NF are oleic acid, linoleic acid and palmitic acid (Appendix D). On average, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids constitute 23.1%, 53.0% and 23.5% of the total fatty acids, respectively (ISO 12966-2/4). The average *trans* fatty acid content is 0.21% of total fatty acids.

The applicant provided analytical data on the levels of some minerals and vitamins (Table 17), and after EFSA's request, added data on boron, molybdenum, and iodine.

Considering the higher concentrations reported in Table 17, the specifications (Table 14) and the estimated P95 of exposure to the NF, the Panel notes that none of the existing upper levels for the analysed micronutrients are expected to be exceeded from the NF intake alone, for any population group.

The Panel noted that the NF is not a significant dietary contributor of vitamin D3. Intake of Mn, for which upper levels have not been established by EFSA, was also considered. The SCF (2000) reported that exposure to high levels of Mn by inhalation or oral intake may be neurotoxic. The SCF could, however, not set a UL for Mn and concluded that 'the margin between oral effect levels in humans, as well as experimental animals and the estimated intake from food, is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to Mn beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit' (SCF/NDA, 2006).

The concentration of Mn in the NF according to specifications (Table 14), may reach 11.5 mg/kg. This concentration is comparable to food sources rich in Mn, e.g. nuts 24.9 mg/kg; dried fruit, nuts and seeds 11.9 mg/kg; chocolate 8.9 mg/kg; bread, miscellaneous cereals 8.0 mg/kg (EFSA NDA Panel, 2013). EFSA estimated the intake of Mn from the NF, considering the product specification for Mn (Table 14) and the estimated daily intake of the NF for all population groups (Table 16). EFSA has previously reported that estimated mean Mn intakes for adults in the EU ranged from 2 to 6 mg/day, with the majority of intake values being around 3 mg/day (EFSA NDA Panel, 2013). In younger age groups, mean Mn intakes in various EU countries ranged from 1.5 to 3.5 mg/day in children, and from 2 to 6 mg/day in adolescents (EFSA NDA Panel, 2013). The highest estimated P95 intake of Mn from the NF ranges from 0.09 mg/day in infants to 0.23 mg/day in adults. As compared to the highest mean background Mn intake estimates, the additional intake of manganese from the NF would be 3.1% for young children, 4.2% for other children, 3.4% for adolescents and 4.0% in adults. The Panel considers that such an increase of Mn intake (< 5% of the highest mean background intake[14]) from

[14] As reported in the published minutes of the 131st meeting of the working group on novel foods (WG NF 2022), the working group (WG) considered that 'for the purpose of the assessment of NFs, intakes that lead to a significant increase of Mn intake as compared to the background diet are considered of concern. The WG also noted that an assessment of UL for Mn is ongoing (EFSA-Q-2021-00371). Based on experts' judgement and criteria set by the WHO/FAO's Codex Alimentarius Commission (2015) for selecting foods/food groups that contribute significantly to total dietary exposure of a contaminant or toxin, the WG concluded that Mn intake above 5% as compared to the high mean background intake (EFSA NDA Panel, 2013) is considered as a significant contribution'.

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

Parameter	Batch number					Analytical method
Minerals (mg/100 g)						
	#1	**#2**	**#3**	**#92**	**#72**	
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	9.81	10.10	9.66	10.70	11.9	
	#93	**#94**	**#95**	**#96**	**#97**	
Molybdenum	0.113	0.119	0.107	0.114	0.109	Internal, ICP-MS[a]
	#93	**#87**	**#98**	**#96**	**#97**	
Iodine	0.27	0.30	0.25	0.25	0.22	DIN EN 15111 (2007–06), mod., ICP-MS[a]
	#86	**#99a**	**#100**	**#99b**	**#101**	
Boron	0.53	0.51	0.47	0.53	0.50	Internal, ICP-MS[a]
	#1	**#2**	**#3**	**#4**	**#5**	
Sodium	135	131	129	141	139	Internal, FAAS[b]
Vitamins						
	#90	**#102**	**#21**	**#103**	**#104**	
Vitamin A (Retinol) (µg/100 g)	< 21.0	< 21.0	< 21.0	< 21.0	< 21.0	EN 12823–1 2014, LC-DAD[c]
	#105	**#2**	**#106**	**#107**	**#3**	
α-Tocopherol (mg/100 g)	1.55	1.58	1.56	1.59	1.13	EN 12822:2014, LC-FLUO[d]
β-Tocopherol (mg/100 g)	< 0.500	< 0.500	< 0.500	< 0.500	< 0.500	
γ-Tocopherol (mg/100 g)	1.19	1.20	1.22	1.19	0.778	
δ-Tocopherol (mg/100 g)	< 0.500	< 0.500	< 0.500	< 0.500	< 0.500	
Vitamin E (Tocopherols) (mg/100 g)	2.74	2.78	2.78	2.78	1.91	
	#108	**#109**	**#110**	**#111**	**#112**	
Vitamin D2 (Ergocalciferol) (µg/100 g)	< 0.250	< 0.250	0.650	< 0.250	0.980	EN 12821:2009, LC-DAD[c]
	#52	**#53**	**#54**	**#3**	**#55**	
Vitamin D3 (Cholecalciferol) (µg/100 g)	57.7	61.2	50.2	51.6	62.5	EN 12821:2009, LC-DAD[c]

(a): ICP-MS: Inductively coupled plasma mass spectrometry.
(b): FAAS: Flame atomic absorption spectroscopy.
(c): LC-DAD: Liquid chromatography/diode array detection.
(d): LC-FLUO: Liquid chromatography/fluorescence.

the NF is not of concern. Additionally, the Panel notes that an increasing number of NFs deriving from *T. molitor* larvae becomes available to the EU consumers. It is expected that the combined intake of such products may increase the intake of manganese from *T. molitor* larvae.

The NF contains on average 6.6 g chitin in 100 g of NF. The Panel considers that chitin is an insoluble fibre that is not expected to be digested in the small intestine of humans to any significant degree, although partial digestion in the human stomach has been suggested (Paoletti et al., 2009;

Muzzarelli et al., 2012). It is also rather resistant to microbial fermentation and therefore assumed to be excreted mainly unchanged. Additionally, the Panel notes that chitin can bind bivalent minerals (Franco et al., 2004; Anastopoulos et al., 2017) possibly affecting their bioavailability, as reported for dietary fibres in general (Baye et al., 2017).

Insects may contain antinutritional factors (ANFs) such as tannins, oxalates, phytates, hydrogen cyanide (Shantibala et al., 2014; Meyer-Rochow et al., 2021), thiaminases (Nishimune et al., 2000) and protease inhibitors (Eguchi, 1993). The applicant determined the concentrations of hydrogen cyanide, oxalic acid, total polyphenols expressed as gallic acid, trypsin inhibitors, phytic acid and tannins in five independently produced batches of the NF (Table 18). The reported values in the NF are comparable to the occurrence levels of these compounds in other foodstuffs (Rao and Prabhavathi, 1982; Gupta, 1987; Holmes and Kennedy, 2000; Schlemmer et al., 2009; EFSA CONTAM Panel, 2019), and also to other published EFSA NF *T. molitor* opinions (EFSA NDA Panel, 2021a,b).

Table 18: Levels of antinutrients in the NF

Parameter	Batch number					Analytical method
	#113	**#114**	**#115**	**#116**	**#117**	
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)

(a): HS-GC-NPD: Headspace gas chromatography/nitrogen–phosphorus detector.
(b): HPLC-IC-EC: High-performance liquid chromatography, ion chromatography/conductivity detection.
(c): UV/VIS: Ultraviolet/visible.
(d): ICP-OES: Inductively coupled plasma - optical emission spectrometry.

The Panel considers that taking into account the composition of the NF and the proposed conditions of use consumption of the NF is not nutritionally disadvantageous.

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.

4. Discussion

The NF which is the subject of the application is the UV-treated powder of whole yellow mealworm (*T. molitor* larva). The production process is sufficiently described and does not raise safety concerns. The Panel considers that the NF is sufficiently characterised. The concentrations of contaminants depend mainly on the occurrence of these substances in the insect feed. The Panel notes that the microbiological values of the analysed samples do not exceed the specification limits. Provided that the respective EU legislation regarding feed is followed, the consumption of the NF does not raise safety concerns regarding contaminants derived from the feed. The Panel notes that there are no safety concerns regarding stability if the NF complies with the proposed specification limits during its entire shelf life.

The applicant intends to market the NF as an ingredient in several food products. The target population is the general population. Intake was estimated based on the use of the NF as an ingredient in the intended food categories at the maximum proposed levels across surveys in the EFSA Comprehensive European Food Consumption Database. The highest intake estimate was calculated for infants (< 1 year old) ranging from 227 to 845 mg NF/kg bw per day at the 95th percentile of the intake distribution. The Panel notes that consumption of the NF under the proposed uses and use levels does not contribute substantially to the total dietary exposure of the population to the analysed undesirable substances (heavy metals, mycotoxins).

The Panel notes that although the protein levels of the NF are overestimated due to the presence of non-protein nitrogen of chitin, the NF has a high content of true protein. None of the existing upper levels for the analysed micronutrients are exceeded considering the proposed uses and use levels. The reported concentrations of antinutritional factors in the NF are comparable to those in other foods. The Panel considers that the main type of fibre in the NF, chitin, is an insoluble fibre not expected to be digested in the small intestine of humans to any significant degree and is assumed to be excreted mainly unchanged. Additionally, the Panel notes that chitin, like other fibres, can possibly affect the bioavailability of minerals. Taking into account the composition of the NF and the proposed conditions of use, the Panel concludes that the consumption of the NF is not nutritionally disadvantageous. Despite the UV treatment, the Panel notes that the NF is not a significant dietary contributor of vitamin D3.

As no adverse effects were observed either in the toxicological studies available in the literature on dried yellow mealworms or were identified from the history of use of the NF and its source, the Panel considers that there are no safety concerns, provided the larvae are reared separately from the adults.

[15] Commission Implementing Regulation (EU) No 828/2014 of 30 July 2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food. OJ L 228, 31.7.2014, p. 5–8.

The Panel considers that the consumption of the NF may induce primary sensitisation and allergic reactions to yellow mealworm proteins and may cause allergic reactions in subjects with allergies to crustaceans and dust mites due to cross-reactivity. Additionally, the Panel notes that allergens from the feed (e.g. gluten) may end up in the NF.

5. Conclusions

The Panel concludes that the NF is safe under the proposed uses and use levels. In addition, the Panel notes that allergic reactions are likely to occur.

5.1. Protection of Proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the following data claimed as proprietary by the applicant: the production process and compositional analyses.

6. Recommendations

As previously recommended by the Panel (EFSA NDA Panel, 2021a,b), research should be undertaken on the allergenicity to yellow mealworm, including cross-reactivity to other allergens.

7. Steps taken by EFSA

1) On 15 June 2020 EFSA received a letter from the European Commission with a request for a scientific opinion on the safety of *Tenebrio molitor* (mealworm) flour. Ref. Ares(2020) 3103859–15/06/2020.
2) On 15 June 2020, a valid application on *Tenebrio molitor* (mealworm) flour, which was submitted by Nutri'Earth, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1142) and the scientific evaluation procedure was initiated.
3) On 23 November 2020, 23 July 2021, 23 November 2021, and 13 May 2022, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 02 July 2021, 12 October 2021, 21 April 2022, and 08 March 2023, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 17 May 2022, EFSA received a letter from the European Commission with the request for a scientific opinion on UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larvae) as a novel food. Ref. Ares (2022)3729832–17/05/2022.
6) During its meeting on 28 March 2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of UV-treated powder of whole yellow mealworm (*Tenebrio molitor* larva) as a NF pursuant to Regulation (EU) 2015/2283.

References

Anastopoulos I, Bhatnagar A, Bikiaris DN and Kyzas GZ, 2017. Chitin adsorbents for toxic metals: a review. International Journal of Molecular Sciences, 18, 114.

Andersen G, Marcinek P, Sulzinger N, Schieberle P and Krautwurst D, 2019. Food sources and biomolecular targets of tyramine. Nutrition Reviews, 77, 107–115.

Attygalle AB, Blankespoor CL, Meinwald J and Eisner T, 1991. Defensive secretion of Tenebrio molitor (Coleoptera: Tenebrionidae). Journal of Chemical Ecology, 17, 805–809.

Baye K, Guyot JP and Mouquet-Rivier C, 2017. The unresolved role of dietary fibers on mineral absorption. Critical Reviews in Food Science and Nutrition, 57, 949–957.

Bednarska AJ and Świątek Z, 2016. Subcellular partitioning of cadmium and zinc in mealworm beetle (Tenebrio molitor) larvae exposed to metal-contaminated flour. Ecotoxicology and Environmental Safety, 133, 82–89.

Brown WV, Doyen JT, Moore BP and Lawrence JF, 1992. Chemical composition and taxonomic significance of defensive secretions of some Australian Tenebrionidae (Coleoptera). Australian Journal of Entomology, 31, 79–89.

Dutta PK, Dutta J and Tripathi VS, 2004. Chitin and chitosan: chemistry, properties and applications.

EFSA (European Food Safety Authority), 2011. Use of the EFSA comprehensive European food consumption database in exposure assessment. EFSA Journal 2011;9(3):2097, 34 pp. https://doi.org/10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2015. The food classification and description system FoodEx2 (revision 2). EFSA Supporting Publication 2015;12(5):EN-804, 90 pp. https://doi.org/10.2903/j.efsa.2015.en-804

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2011. Scientific Opinion on Scientific Opinion on risk-based control of biogenic amine formation in fermented foods. EFSA Journal 2011;9(10):2393, 93 pp. https://doi.org/10.2903/j.efsa.2011.2393

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Leblanc J-C, Nebbia CS, Nielsen E, Ntzani E, Petersen A, Sand S, Vleminckx C, Wallace H, Benford D, Brimer L, Mancini FR, Metzler M, Viviani B, Altieri A, Arcella D, Steinkellner H and Schwerdtle T, 2019. Scientific opinion on the evaluation of the health risks related to the presence of cyanogenic glycosides in foods other than raw apricot kernels. EFSA Journal 2019;17(4):5662, 78 pp. https://doi.org/10.2903/j.efsa.2019.5662

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2012. Scientific Opinion on Dietary Reference Values for protein. EFSA Journal 2012;10(2):2557, 66 pp. https://doi.org/10.2903/j.efsa.2012.2557

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on Dietary Reference Values for manganese. EFSA Journal 2013;11(11):3419, 44 pp. https://doi.org/10.2903/j.efsa.2013.3419

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Ververis E and Knutsen HK, 2021a. Scientific Opinion on the safety of dried yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(1):6343, 29 pp. https://doi.org/10.2903/j.efsa.2021.6343

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Ververis E and Knutsen HK, 2021b. Scientific Opinion on the safety of frozen and dried formulations from whole yellow mealworm (*Tenebrio molitor* larva) as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(8):6778, 30 pp. https://doi.org/10.2903/j.efsa.2021.6778

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Maradona MP, Schlatter JR, van Loveren H, Azzollini D and Knutsen HK, 2021c. Scientific Opinion on the safety of frozen and dried formulations from migratory locust (Locusta migratoria) as a Novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(7):6667, 30 pp. https://doi.org/10.2903/j.efsa.2021.6667

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Bohn T, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Prieto Maradona M, Schlatter JR, van Loveren H, Goumperis T and Knutsen HK, 2021d. Scientific Opinion on the safety of frozen and dried formulations from whole house crickets (Acheta domesticus) as a Novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2021;19(8):6779, 29 pp. https://doi.org/10.2903/j.efsa.2021.6779

EFSA Scientific Committee, 2015. Risk profile related to production and consumption of insects as food and feed. EFSA Journal 2015;13(10):4257, 60 pp. https://doi.org/10.2903/j.efsa.2015.4257

Eguchi M, 1993. Protein protease inhibitors in insects and comparison with mammalian inhibitors. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 105, 449–456.

FAO (Food and Agriculture Organization), 2013. Dietary protein quality evaluation in human nutrition: report of an FAO Expert Consultation. Food and nutrition paper; 92. Rome, FAO.

Feng Y, Chen XM, Zhao M and Ding WF, 2018. Edible insects in China: utilization and prospects. Insect Science, 25, 184–198.

Finke MD, 2007. Estimate of chitin in raw whole insects. Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association, 26, 105–115.

Franco LDO, Maia RDCC, Porto ALF, Messias AS, Fukushima K and Campos-Takaki GMD, 2004. Heavy metal biosorption by chitin and chitosan isolated from Cunninghamella elegans (IFM 46109). Brazilian Journal of Microbiology, 35, 243–247.

Ghannem S, Touaylia S and Boumaiza M, 2018. Beetles (Insecta: Coleoptera) as bioindicators of the assessment of environmental pollution. Human and Ecological Risk Assessment: An International Journal, 24, 456–464.

Gupta YP, 1987. Anti-nutritional and toxic factors in food legumes: a review. Plant Foods for Human Nutrition, 37, 201–228.

Hahn T, Roth A, Febel E and Zibek S, 2018. New methods for high-accuracy insect chitin measurement. Journal of the Science of Food and Agriculture, 98, 5069–5073.
Han X and Heinonen M, 2020. Development of ultra-high-performance liquid chromatographic and fluorescent method for the analysis of insect chitin. Food Chemistry, 334, 127577.
Han SR, Yun EY, Kim JY and Moon KS, 2014. Evaluation of genotoxicity and 28-day oral dose toxicity on freeze-dried powder of tenebrio molitor larvae (Yellow Mealworm). Toxicological Research, 30, 121–130.
Han SR, Lee BS, Jung KJ and Moon KS, 2016. Safety assessment of freeze-dried powdered Tenebrio molitor larvae (yellow mealworm) as novel food source: evaluation of 90-day toxicity in Sprague-Dawley rats. Regulatory Toxicology and Pharmacology, 77, 206–212.
Hanboonsong Y, Jamjanya T. and Durst PB, 2013. Six-legged livestock: edible insect farming, collection and marketing in Thailand.
Holmes RP and Kennedy M, 2000. Estimation of the oxalate content of foods and daily oxalate intake. Kidney International, 57, 1662–1667.
Houbraken M, Spranghers T, De Clercq P and Spanoghe P, 2016. Pesticide contamination of Tenebrio molitor (Coleoptera: Tenebrionidae) for human consumption. Food Chemistry, 201, 264–269.
Janssen RH, Lakemond CM, Fogliano V and Vincken JP, 2017a. Involvement of phenoloxidase in browning during grinding of Tenebrio molitor larvae. PloS One, 12, e0189685.
Janssen RH, Vincken JP, van den Broek LA, Fogliano V and Lakemond CM, 2017b. Nitrogen-to-protein conversion factors for three edible insects: Tenebrio molitor, Alphitobius diaperinus, and Hermetia illucens. Journal of Agricultural and Food Chemistry, 65, 2275–2278.
Komi D, Sharma L and Dela Cruz CS, 2018. Chitin and its effects on inflammatory and immune responses. Clinical Revenue Allergy Immunology, 54, 213–223.
Kooh P, Ververis E, Tesson V, Boué G and Federighi MM, 2019. Entomophagy and public health: a review of microbiological hazards. Health, 11, 1272–1290.
Kumirska J, Weinhold MX, Czerwicka M and Stepnowski P, 2011. Influence of the chemical structure and physicochemical properties of chitin-and chitosan-based materials on their biomedical activity. Biomedical Engineering, Trends in Materials Science, 25–64.
Ladisch RK, Ladisch SK and Howe PM, 1967. Quinoid secretions in grain and flour beetles. In Proceedings of the Pennsylvania Academy of Science (pp. 213–219). Pennsylvania Academy of Science.
Lindqvist L and Block M, 1995. Excretion of cadmium during moulting and metamorphosis in Tenebrio molitor (Coleoptera; Tenebrionidae). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 111, 325–328.
Meyer-Rochow VB, Gahukar RT, Ghosh S and Jung C, 2021. Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stage, gender, diet, and processing method. Foods, 10, 1036.
Muñoz-Esparza NC, Latorre-Moratalla ML, Comas-Basté O, Toro-Funes N, Veciana-Nogués MT and Vidal-Carou MC, 2019. Polyamines in Food. Frontiers of Nutrition, 11, 108.
Muthukrishnan S, Merzendorfer H, Arakane Y and Yang Q, 2016. Chitin metabolic pathways in insects and their regulation. Extracellular composite matrices in arthropods. Springer, Cham. pp. 31–65.
Muzzarelli RA and Raa M, 1973. Natural chelating polymers. Alginic acid, chitin and chitosan.
Muzzarelli RA, Jeuniaux C and Gooday GW, 1986. Chitin in nature and technology. 385. Plenum Press, New York.
Muzzarelli RA, Boudrant J, Meyer D and Paoletti MG, 2012. Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: a tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. Carbohydrate Polymers, 87, 995–1012.
Nappi AJ and Christensen BM, 2005. Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. Insect Biochemical Molecular Biology, 35, 443–459.
Nappi AJ and Ottaviani E, 2000. Cytotoxicity and cytotoxic molecules in invertebrates. BioEssays, 22, 469–480. https://doi.org/10.1002/(SICI)1521-1878(200005)22:5<469:AID-BIES9>3.0.CO;2-4
Nappi AJ and Vass E, 1993. Melanogenesis and the generation of cytotoxic molecules during insect cellular immune-reactions. Pigment Cell Research, 6, 117–126. https://doi.org/10.1111/j.1600-0749.1993.tb00590.x
Nishimune T, Watanabe Y, Okazaki H and Akai H, 2000. Thiamin is decomposed due to Anaphe spp. entomophagy in seasonal ataxia patients in Nigeria. The Journal of Nutrition, 130, 1625–1628.
Panagiotakopulu E, 2000. Archaeology and entomology in the Eastern Mediterranean. BAR International Series, 836.
Panagiotakopulu E, 2001. New records for ancient pests: archaeoentomology in Egypt. Journal of Archaeological Science, 28, 1235–1246.
Paoletti MG, Norberto L, Cozzarini E and Musumeci S, 2009. XX role of chitinases in human stomach for chitin digestion: AMCase in the gastric digestion of chitin and chit in gastric pathologies.
Ramos-Elorduy J, 1997. Insects: a sustainable source of food? Ecology of Food and Nutrition, 36, 247–276.
Ramos-Elorduy J, 2009. Anthropo-entomophagy: cultures, evolution and sustainability. Entomological Research, 39, 271–288.
Ramos-Elorduy J and Moreno JMP, 2004. Los coleoptera comestibles de México. Anales del Instituto de Biología. Serie Zoología, 75, 149–183.

Rao BSN and Prabhavathi T, 1982. Tannin content of foods commonly consumed in India and its influence on ionisable iron. Journal of the Science of Food and Agriculture, 33, 89–96.
Roberts GA, 1992. Chitin chemistry. Macmillan International Higher Education.
SCF (Scientific Committee on Food), 2000. Opinion of the Scientific Committee on Food on the tolerable upper intake level of manganese. SCF/CS/NUT/UPPLEV/21 Final, 11.
SCF/NDA (Scientific Committee on Food/Panel on Dietetic Products, Nutrition and Allergies), 2006. Tolerable upper intake levels for vitamins and minerals. Available online: https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf
Schlemmer U, Frølich W, Prieto RM and Grases F, 2009. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Molecular Nutrition and Food Research, 53(S2), S330–S375.
Shantibala T, Lokeshwari RK and Debaraj H, 2014. Nutritional and antinutritional composition of the five species of aquatic edible insects consumed in Manipur. India. Journal of Insect Science, 14.
Sugumaran M, Nellaiappan K and Valivittan K, 2000. A new mechanism for the control of phenoloxidase activity: inhibition and complex formation with quinone isomerase. Archives of Biochemistry and Biophysics, 379, 252–260.
Temova Ž and Roškar R, 2016. Stability-indicating HPLC-UV method for vitamin D3 determination in solutions, nutritional supplements and pharmaceuticals. Journal of Chromatography Science, 54, 1180–1186.
Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N and Peterson RE, 2006. The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. Toxicological Sciences, 93, 223–241. https://doi.org/10.1093/toxsci/kfl055
Van der Fels-Klerx HJ, Camenzuli L, Van Der Lee MK and Oonincx DGAB, 2016. Uptake of cadmium, lead and arsenic by Tenebrio molitor and Hermetia illucens from contaminated substrates. PloS One, 11, e0166186.
Vandeweyer D, De Smet J, Van Looveren N and Van Campenhout L, 2021. Biological contaminants in insects as food and feed. Journal of Insects as Food and Feed, 7, 807–822.
Vigneron A, Masson F, Vallier A and Heddi A, 2014. Insects recycle endosymbionts when the benefit is over. Current Biology, 24, 2267–2273. https://doi.org/10.1016/j.cub.2014.07.065
Vijver M, Jager T, Posthuma L and Peijnenburg W, 2003. Metal uptake from soils and soil–sediment mixtures by larvae of Tenebrio molitor (L.) (Coleoptera). Ecotoxicology and Environmental Safety, 54, 277–289.
Wacker M and Holick MF, 2013. Sunlight and Vitamin D: a global perspective for health. Dermatoendocrinology, 5, 51–108.
Wittig M, Krings U and Berger R, 2013. Single-run analysis of vitamin D photoproducts in oyster mushroom (Pleurotus ostreatus) after UV-B treatment. Journal of Food Composition and Analysis., 31, 266–274.

Abbreviations

3-MCPD	3-monochloropropane-1,2-diol
ADF	acid detergent fibre
ADL	acid detergent lignin
ADME	absorption, distribution, metabolism and excretion
ANF	antinutritional factors
AOAC	Association of Official Analytical Chemists
ASU	Official Collection of Analysis Methods According To § 64 of the German Food And Feed Code (LFGB)
a_w	water activity
BIOHAZ	EFSA Panel on Biological Hazards
BRD	Bacteriology Reference Department
bw	body weight
CFU	colony forming units
CLA	conjugated linoleic acid
CONTAM	EFSA Panel on Contaminants in The Food Chain
DIAAS	Digestible Indispensable Amino Acid Score
DIN	Deutsches Institut Fur Normung (German Institute for Standardisation)
DRVs	dietary reference values
ELISA	enzyme-linked immunosorbent assay
EN	Europaische Norm (European Standard)
FAAS	flame atomic absorption spectrometry
FAO	Food and Agriculture Organization of The United Nations
FFA	free fatty acids

GC-FID	gas chromatography with flame- detection
GC–MS/MS	gas chromatography with tandem mass spectrometry
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HPLC-DAD	high-performance liquid chromatography with diode array detection
HS-GC-NPD	head-space gas chromatography with nitrogen-phosphorus detection
IAA	indispensable amino acids
IAC-LC-FLD	immunoaffinity chromatography- liquid chromatography with fluorescence detector
IC-EC	ion chromatography/conductivity detection
IC-PAD	ion chromatography-pulsed amperometric detection
ICP-MS	inductively coupled plasma-mass spectrometry
ICP-OES	inductively coupled plasma atomic emission spectroscopy
IC-UV	ion chromatography/ ultraviolet detection
ISO	International Organization for Standardization
IU	International Units
K-PDCAAS	protein digestibility assay kit
LC-FLUO	liquid chromatography-fluorescence detection
LC-DAD	liquid chromatography-diode array detection
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LC-UV/DAD	liquid chromatography-ultraviolet diode array detection
LOD	limit of detection
LOQ	limit of quantification
meq	milliequivalent
MLs	maximum levels
Mn	manganese
MUFA	mono-unsaturated fatty acids
ND	not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
nr	not reported
PA	*p*-anisidine value
PCBs	polychlorinated biphenyls
PCR	polymerase chain reaction
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PRI	population reference intakes
PUFA	poly-unsaturated fatty acids
PV	peroxide value
SCF	Scientific Committee on Food
SFA	saturated fatty acids
UB	upper bound
UV- VIS	ultraviolet–visible
w/w	weight per weight
WHO	World Health Organization
WHO (2005)	sum of polychlorinated dibenzo-*p*-dioxins-polychlorinated
PCDD/F + PCB TEQ	dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent

Appendix A – Proximate analysis of yellow mealworm powder before and after UV treatment

Parameter (unit)	Batch number										Analytical method
	Before UV treatment					After UV treatment (NF)					
	*(#140)	*(#111)	*(#130)	*(#141)	*(#132)	#1	#2	#3	#4	#5	
Crude protein (g/100 g of NF)	53.3	52.8	52.4	53.7	53.7	53.1	52.5	52.3	53.6	53.7	Kjeldahl ($N \times 6.25$)
Crude fat (g/100 g of NF)	33.6	33.8	33.7	33.4	33.4	34.1	33.9	35.3	33.9	33.9	Internal, Gravimetric
Total carbohydrates (g/100 g of NF)	6.0	6.5	6.9	5.8	6.1	6.2	7.2	6.1	6.9	6.5	Calculation by difference
Of which sugar (g/100 g of NF)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	Internal, IC-PAD[(a)]
Ash (g/100 g of NF)	3.7	3.6	3.6	3.6	3.6	3.5	3.5	3.4	3.8	3.9	Internal, Gravimetric
Moisture (g/100 g of NF)	3.4	3.2	3.4	3.4	3.2	3.0	2.9	2.9	1.9	2.0	Internal Thermogravimetric
Energy (kcal/100 g of NF)	540	542	540	539	539	545	544	552	547	546	Regulation (EU) 1169/2011
Energy (kJ/100 g of NF)	2,252	2,260	2,254	2,249	2,251	2,272	2,270	2,300	2,281	2,278	Regulation (EU) 1169/2011

NF: novel food.
(a): IC-PAD: ion chromatography-pulsed amperometric detection.
*: These are not NF batches.

Appendix B – Biogenic amines levels of the NF during the proposed shelf life (t = 6 months)

Parameter (mg/kg)	Batch number					Analytical method
	#71	#30	#5	#27	#135	
Cadaverine	7.42	9.33	9.86	9.69	9.57	Czech J. Food Sci. Vol. 21, LC-UV/DAD[(a)]
Spermidine	65.8	3.58	3.55	170	168	
Spermine	18.3	65.3	62.2	68.4	68.2	
Histamine	< 1	< 1	< 1	< 1	< 1	
Putrescine	287	468	477	481	468	
Tyramine	1.61	2.54	1.71	1.93	2.66	
Tryptamine	< 5	< 5	< 5	< 5	< 5	
2-Phenylethylamine	< 1	< 1	< 1	< 1	< 1	

NF: novel food.
(a): LC-UV/DAD: Liquid chromatography-ultraviolet detection/diode array detection.

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					UV-treated (NF)				
	*(#9)	*(#10)	*(#87)	*(#125)	*(#127)	#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	6.22	5.9	5.87	6.07	6.24	6.1	6.17	6.15	6.22
Glycine[1]	2.87	2.82	2.89	2.84	2.93	2.85	2.71	2.83	2.88	2.84
Histidine**[1]	1.66	1.68	1.7	1.67	1.69	1.69	1.61	1.69	1.69	1.66
Hydroxyproline[1]	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Isoleucine**[1]	2.29	2.3	2.28	2.31	2.29	2.31	2.2	2.29	2.27	2.3
Leucine**[1]	3.95	3.97	3.89	3.94	4.02	3.99	3.91	3.95	3.98	3.97
Lysine**[1]	3.14	3.28	3.11	3.09	3.07	3.26	3.14	3.24	3.21	3.24
Methionine**[1]	0.696	0.711	0.697	0.67	0.69	0.698	0.687	0.725	0.701	0.7
Ornithine[1]	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Phenylalanine**[1]	1.93	2.01	1.93	1.94	1.97	2.01	1.97	2	2	2
Proline[1]	3.43	4	3.44	3.53	3.51	4.02	3.92	4.08	4.3	4.11
Serine[1]	2.39	2.5	2.49	2.53	2.66	2.51	2.48	2.51	2.55	2.49
Threonine**[1]	2.06	2.16	2.12	2.17	2.25	2.15	2.24	2.16	2.16	2.15
Tryptophan**[2]	0.6	0.592	0.608	1.84	0.579	0.58	0.585	0.577	0.552	0.515
Tyrosine[1]	3.79	3.86	3.89	3.88	3.93	3.81	3.73	3.83	3.95	3.84
Valine**[1]	3.33	3.26	3.3	3.3	3.34	3.26	3.12	3.23	3.24	3.24

NF; novel food.
*: These are not NF batches.
**: Indispensable amino acids; LOQ: limit of quantification.
1: ISO 13903:2005, IC-UV (Ion Chromatography-Ultraviolet detection).
2: Commission Regulation (EC) 152/2009, LC-FLUO (liquid chromatography-fluorescence).

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)	Before UV treatment					After UV treatment (NF)				
	* (#140)	* (#111)	* (#130)	* (#141)	* (#132)	#1	#2	#3	#4	#5
Total Saturated (SFA)	7.61	7.75	7.73	7.74	7.72	7.61	7.60	7.90	7.34	7.28
C4:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C6:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C7:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C8:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.03	0.04
C9:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C10:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C11:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C12:0	0.10	0.10	0.11	0.12	0.11	0.12	0.12	0.12	0.12	0.13
C13:0	< 0.01	< 0.01	< 0.01	< 0.01	0.02	< 0.01	0.02	< 0.01	0.02	0.02
C14:0	1.19	1.22	1.22	1.22	1.24	1.30	1.32	1.33	1.41	1.36
C15:0	0.04	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.03
C16:0	5.23	5.30	5.28	5.27	5.26	5.24	5.21	5.41	4.86	4.82
C17:0	0.04	0.14	0.13	0.14	0.14	0.03	0.03	0.14	0.03	0.02
C18:0	0.90	0.90	0.91	0.90	0.88	0.84	0.82	0.85	0.78	0.8
C19:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:0	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.04	0.04	0.04
C21:0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:0	0.04	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	0.02
C24:0	0.04	< 0.01	< 0.01	< 0.01	< 0.01	0.03	0.03	< 0.01	< 0.01	< 0.01
Total monounsaturated (MUFA)	16.00	16.02	15.93	15.77	15.70	17.08	16.93	17.84	17.49	17.45
C11:1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C12:1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C13:1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C14:1 (n-5c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C14:1 (n-5 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C15:1 (n-5c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C15:1 (n-5 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C16:1 (n-7c)	0.71	0.73	0.72	0.71	0.72	0.79	0.80	0.79	0.84	0.81
C16:1 (n-7 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C17:1 (n-7c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C17:1 (n-7 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:1 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:1 (n-7c)	0.09	0.10	0.09	0.10	0.09	0.10	0.09	0.09	0.08	0.08
C18:1 (n-7 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:1 (n-9c)	15.16	15.17	15.08	14.92	14.84	16.15	16.01	16.93	16.55	16.52
C18:1 (n-9 t) + C18:1 (n-12 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C19:1 (n-12 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C19:1 (n-9 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:1 (n-9c)	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.02	0.03	0.04
C22:1 (n-11)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:1 (n-9c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:1 (n-9 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C24:1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Fatty acids (g/100 g NF)	Before UV treatment					After UV treatment (NF)				
	* (#140)	* (#111)	* (#130)	* (#141)	* (#132)	#1	#2	#3	#4	#5
Total polyunsaturated (PUFA)	8.45	8.49	8.44	8.39	8.41	7.84	7.78	7.93	7.42	7.52
C18:2 (9c, 11 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:2 (n-6c)	8.16	8.19	8.12	8.07	8.10	7.56	7.52	7.72	7.16	7.26
C18:2 (n-6 t)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:2 t2	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.08	0.06	0.05
C20:2 (n-6c)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	< 0.01	0.02	0.02
C22:2 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:3 (n-3)	0.27	0.28	0.28	0.28	0.28	0.23	0.22	0.22	0.22	0.21
C18:3 (n-6)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:3 t3 (C18:3 t1 + C18:3 t2)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:3 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:3 (n-6c)	< 0.01	< 0.01	0.02	0.02	0.02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C18:4 (n-3)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C20:4 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.03	0.03	< 0.01	< 0.01	< 0.01
C20:5 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:5 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:5 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C22:6 (n-3c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
C 22:3 (n-3c) + C22:4 (n-6c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.03	0.03
C20:1 (n-9 t) + C18:2 (10 t, 12c) + C20:1 (n-15c)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Total *Trans*	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.08	0.06	0.05
Total Omega-3	0.27	0.28	0.28	0.28	0.28	0.23	0.22	0.22	0.22	0.21
Total Omega-6	8.16	8.19	8.12	8.07	8.10	7.59	7.54	7.72	7.16	7.26
Other	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fatty acids Omega-6/ Omega-3 Ratio	29.81	29.60	29.05	28.41	28.68	33.06	34.18	35.18	33.2	34.35

NF: novel food.
*: These are not NF batches.

Annex A – Dietary exposure estimates to the Novel Food for each population group from each EU dietary survey

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.8009#support-information-section).