

Safety of frozen, dried and powder forms of house crickets (*Acheta domesticus*) as a novel food pursuant

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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 the safety of frozen, dried and powder forms of house crickets (*Acheta domesticus*) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is proposed in three forms: (i) frozen, (ii) dried, (iii) powder. The main components of the NF are protein, fat and dietary fibre (chitin). The Panel notes that the concentration of contaminants in the NF depends on the occurrence levels of these substances in the insect feed. The NF has a protein content that ranges between 19.7 and 20.9 g/100 g in the frozen form and 61.7–68.6 g/100 g in the dried and powder forms. The Panel acknowledges that the true protein content is overestimated when using the nitrogen-to-protein conversion factor of 6.25 due to the presence of non-protein nitrogen from chitin. The applicant proposed to use the NF as food ingredient in a number of food products. The target population proposed by the applicant is the general population. Considering the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The Panel notes that no safety concerns arise from the toxicological information on *A. domesticus*. The Panel considers that the consumption of the NF might trigger primary sensitisation to *A. domesticus* proteins and may cause allergic reactions in subjects allergic to crustaceans, mites and molluscs. Additionally, allergens from the feed may end up in the NF. The Panel concludes that the NF is safe under the proposed uses and use levels.

KEYWORDS

Acheta domesticus, food safety, house cricket, novel food

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1 | INTRODUCTION

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

On 30 April 2018, the Belgian Insect Industry Federation submitted a request to the European Commission in accordance with Article 10 of the Regulation (EU) 2015/2283¹ to authorise the placing on the market of frozen and dried formulations from whole house crickets (*Acheta domestica*) as a novel food.

The applicant requests to authorise the frozen and dried formulations from whole house crickets in a number of foods intended for the general population.

The applicant has not requested data protection under Article 26 of Regulation (EU) 2015/2283.

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 the safety of frozen and dried formulations from whole house crickets (*Acheta domestica*) as a novel food.

1.2 | Interpretation of the Terms of Reference

In the process of the evaluation of this novel food (NF), it became apparent that the term 'formulation' can be considered as the activity of creating, designing or developing food products with an objective of providing some functionality. On that basis, the title of the Opinion was amended to 'frozen, dried and powder forms of house crickets (*Acheta domestica*) as a novel food'.

1.3 | Additional information

On 7 July 2021, the NDA Panel adopted an opinion on the safety of frozen and dried formulations from whole house crickets (*A. domestica*) as an NF pursuant to Article 10 of Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels. Following a favourable opinion by the Standing Committee on Plants, Animals, Food and Feed, the European Commission adopted on 10 February 2022 Commission Implementing Regulation (EU) 2022/188 authorising the placing on the market of frozen, dried and powder forms of *A. domestica* as an NF under Regulation (EU) 2015/2283, and amending Commission Implementing Regulation (EU) 2017/2470.

On 23 March 2022, the NDA Panel adopted an opinion on the safety of partially defatted house cricket (*A. domestica*) powder as an NF pursuant to Article 10 of Regulation (EU) 2015/2283. The Panel concluded that the NF is safe for human consumption under the proposed uses and use levels. The European Commission adopted on 3 January 2023 Commission Implementing Regulation (EU) 2023/5 authorising the placing on the market of *Acheta domestica* (house cricket) partially defatted powder as an NF and amending Commission Implementing Regulation (EU) 2017/2470.

On 26 June 2024, the NDA Panel adopted an opinion on the safety of *Acheta domestica* powder as an 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.

2 | DATA AND METHODOLOGIES

2.1 | Data

The safety assessment of this NF is based on data supplied in the application, information submitted by the applicant following EFSA request(s) for supplementary information and additional data identified by the Panel.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469.²

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all 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 does not include a request for the protection of proprietary data.

¹Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001.

²Commission 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.

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.

In the context of this opinion, EFSA's definition of dietary fibre (i.e. non-digestible carbohydrates plus lignin; EFSA NDA Panel, 2010) does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence as laid down in Annex I of Regulation (EC) 1169/2011³ for:

- a. edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and,
- b. edible synthetic carbohydrate polymers.

It is out of the scope of this opinion to establish whether the fraction of non-digestible carbohydrates present in the NF (chitin) meets the legal definition of dietary fibre in the EU or not.

This assessment concerns only the risks that might be associated with 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 which is the subject of the application is related to different forms of *A. domesticus* (house crickets). 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 house crickets. The frozen form consists mainly of water, crude protein, fat and non-digestible carbohydrates, whereas the dried forms (dried and powder) consist mainly of crude protein, fat and non-digestible carbohydrates.

The NF is proposed to be used as the whole frozen or whole dried insect, or in the form of powder, added as an ingredient to various food products. Products with the NF can be consumed by the general population.

3.2 | Identity of the NF

The NF consists of frozen, dried and powder forms of adult *A. domesticus*, also known as 'house crickets'. *A. domesticus* is an insect species that belongs to the family of *Gryllidae*, subfamily *Gryllinae*, genus *Acheta* (Linnaeus, 1758).

The species lives in the wild in various regions worldwide, including Australia, Asia, Africa, North America and Europe (GBIF, 2022). The identity of the insect species has been certified through meticulous morphological identification techniques. Complementing this, the applicant has substantiated the species identity utilising molecular techniques, specifically DNA analysis employing two mitochondrial DNA markers: the mitochondrial cytochrome C oxidase subunit 1 (COI or Cox1) and the mitochondrial ribosomal RNA, 16S rRNA (*rrnL*).

The NF is intended to be marketed as (A) blanched and frozen *A. domesticus* (AD frozen); (B) blanched and dried *A. domesticus* (AD dried); (C) blanched, dried and ground *A. domesticus* (AD powder).

3.3 | Production process

According to the information provided, the NF is produced in line with Hazard Analysis Critical Control Points (HACCP) principles. The applicant stated that insects were reared and processed based on the specifics developed by the company ALBInsecta and certified in accordance with ISO 22000. The production process can be divided into three distinctive parts: farming, harvesting and post-harvest processing.

Farming includes reproduction of the adult insect population and rearing of the nymphs into adults. The eggs are separated from the adult insects, so that nymphs can consequently grow separately. After being hatched from the eggs, the nymphs grow under monitored temperature and humidity conditions. Harvesting is performed from 35 to 120 days after hatching depending on the rearing conditions. Insects are grown in regularly disinfected containers made of

³Regulation (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 Text with EEA relevance.

certified food-contact polyethylene and polypropylene carbonate. This reduces the probability of plastic ingestion by the *A. domesticus* (EFSA NDA Panel, 2021, 2022). Food-grade cardboard or paper pulp are also used during rearing. The applicant reported that no pesticides, antibiotics, solvents and growth hormones are used during farming. Any insect pests are combatted using UV lamps, pheromone traps or biological control (e.g. parasitic wasps).

The applicant reported that the feed administered to the insects consists of plant-based materials and fishmeal, GMP+ Feed Safety Assurance (GMP+ FSA) certified. The total feed mix is in compliance with Regulation (EC) No 999/2001⁴ which allows fishmeal to be used as an ingredient for farming non-ruminant farmed animals.

During farming, *A. domesticus* can be affected by pathogens including cricket paralysis virus (CrPV) from the *Dicistroviridae* family, the cricket *densovirus* (AdDV) from the *Parvoviridae* family (Maciel-Vergara & Ros, 2017), the *Penaeus merguensis* densovirus (PmergDENV) (La Fauce & Owens, 2008) and the nematode *Heterorhabditis georgiana* (Shapiro-Ilan et al., 2009). Control of rearing conditions is put in place to prevent and control potential infections. Control of mites is performed by biological control. The above literature indicates that these pathogens are specific to insects and non-pathogenic for humans or other vertebrates.

Examples of food-borne bacteria that may be present in *A. domesticus* include *Citrobacter* spp., *Klebsiella* spp. and *Yersinia* spp. (Fernandez-Cassi et al., 2020). However, their potential presence in the NF is monitored by microbiological analysis of *Enterobacteriaceae*.

Adult insects are harvested (35–120 days old) after being separated from the substrate and faeces. Decayed insects are removed after visual inspection. After the harvest, a 24-h fasting step is implemented to allow the insects to discard their bowel content.

The post-harvest processing includes the termination of the insects in hot water. As an alternative for blanching water, steam can be used to reach 105°C during 60 s in the core of insects. The thermal treatment contributes to the reduction of the microbial load of the insects as well as the reduction of enzymatic activity. A cooling down step is performed by rinsing the blanched crickets with cold water. Three forms of the NF are then obtained by processing the whole insects.

The form 'AD frozen' is obtained by deep-freezing the terminated insects at –20°C to reach at least –18°C at the core of the insects in < 5 h and storing at –18°C. The form 'AD dried' is obtained by drying the insects in forced hot-air ventilation ovens up to 12 h at 70°C to reach a water activity below 0.6. The form 'AD powder' is obtained by grinding and sieving the AD dried form to obtain a powder with a granularity below 250 µm. The forms of the NF are stored in sealed packaging at –18°C (AD frozen) or at room temperature (AD dried, AD powder).

The Panel considers that the production process is sufficiently described.

3.4 | Compositional data

The applicant provided analytical information on chemical and microbiological parameters for different batches of the NF forms (i.e. AD frozen; AD dried; AD powder). For all parameters, at least five independently produced batches were analysed, except where otherwise specified. Certificates of accreditation from the laboratories that conducted the analyses were provided by the applicant. Analytical data were produced using methods adapted from other types of matrices. Whenever in-house methods were employed, a full description of the method as well as results of the respective validation procedures have been provided.

It should be noted that the NF is a 'whole food' as defined by the EFSA Scientific Committee (2011), meaning that not all its constituents need to be fully identified and/or characterised (EFSA NDA Panel, 2016).

AD frozen consists mainly of water, crude protein, fat and small amounts of dietary fibre (chitin). In the dried forms (AD dried, AD powder), the concentration of all components, excluding water, are higher, compared to AD frozen due to the reduced water content. The results of the proximate analyses of the NF forms are presented in Tables 1–3. The amino acid, fatty acid, vitamin and mineral composition are reported in Section 3.9 'Nutritional information'.

⁴Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

TABLE 1 Batch-to-batch analysis of the NF AD frozen.

Parameter (g/100 g of NF)	Batch numbers						Analytical method
	#2	#3	#4	#5	#6	#41	
Crude protein	20.8	20.9	20.8	20.8	/	19.7	Kjeldahl (N x 6.25) (adapted from Regulation (EC) No 152/2009)
Fat	5.9	7.1	6.4	6.8	4.2	6.3	Gravimetry (adapted from Regulation (EC) No 152/2009)
Total carbohydrates	0.6	0.5	0.3	<0.1	/	0.1	Calculation (100-moisture, protein, fat, ash, fibre)
Dietary fibre ^a	1.3	2.0	2.1	2.2	/	2.0	Enzymatic-gravimetry (AOAC ^b 985.29)
Ash	1.4	1.4	1.4	1.4	/	1.3	Thermo-gravimetry (adapted from Regulation (EC) No 152/2009)
Moisture	70	68	69	69	/	76	Thermo-gravimetry (adapted from Regulation (EC) No 152/2009)
Energy (kcal/100 g of NF)	142	154	146	149	/	122	Calculation (Regulation (EU) 1169/2011)

Abbreviation: /, not provided; EC, European Commission; EU, European Union; N, Nitrogen.

^aThe term is used as synonymous of non-digestible carbohydrates and does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence laid down in Annex I of Regulation (EC) 1169/2011.

^bAssociation of Official Agricultural Chemists.

TABLE 2 Batch-to-batch analysis of the NF AD dried.

Parameter (g/100 g of NF)	Batch numbers							Analytical method
	#11	#12	#13	#14	#15	#16	#49	
Crude protein	/	62.7	63.7	63.3	64.0	/	61.7	Kjeldahl (N x 6.25) (adapted from Regulation EC 152/2009)
Fat	/	15.9	15.0	15.2	14.7	23.5	18.9	Gravimetry (adapted from Regulation (EC) No 152/2009)
Total carbohydrates	/	1.7	0.5	0.5	<0.1	/	8.8*	Calculation (100-moisture, protein, fat, ash, fibre)
Dietary fibre ^a	5.5	6.3	7.3	7.1	8.0	/		Enzymatic-gravimetry (AOAC ^b 985.29)
Ash	/	5.2	5.2	5.3	5.1	/	4.5	Thermo-gravimetry (adapted from Regulation (EC) No 152/2009)
Moisture	/	8.3	8.4	8.6	8.4	/	6.1	Thermo-gravimetry (adapted from Regulation (EC) No 152/2009)
Energy (kcal/100 g of NF)	/	413	406	406	405	/	452	Regulation (EU) 1169/2011

Abbreviations: /, not provided; EC, European Commission; EU, European Union; N, Nitrogen.

^aThe term is used as synonymous of non-digestible carbohydrates and does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence laid down in Annex I of Regulation (EC) 1169/2011.

^bAssociation of Official Agricultural Chemists.

*100- (fat+moisture+ash+protein) fibre were not measured and subtracted in this batch.

TABLE 3 Batch-to-batch analysis of the NF AD powder.

Parameter (g/100 g of NF)	Batch numbers							Analytical method
	#21	#22	#23	#24	#25	#59	#63	
Crude protein	68.5	68.5	68.6	68.3	68.6	67.1	66.8	Kjeldahl (N x 6.25) (adapted from Regulation (EC) No 152/2009)
Fat	12.1	12.2	12.2	12.4	12.2	17.8	16.7	Gravimetry (adapted from Regulation (EC) No 152/2009)
Total carbohydrates	0.8	3.7	0.8	<0.1	2.1	/	/	Calculation (100-moisture, protein, fat, ash, fibre)
Dietary fibre ^a	8.4	5.4	8.4	9.1	6.7	/	/	Enzymatic-gravimetry (AOAC 985.29)

TABLE 3 (Continued)

Parameter (g/100 g of NF)	Batch numbers							Analytical method
	#21	#22	#23	#24	#25	#59	#63	
Ash	4.5	4.6	4.6	4.6	4.6	/	/	Thermo-gravimetry (adapted from Regulation (EC) No 152/2009)
Moisture	5.8	5.5	5.5	5.6	5.8	/	/	Thermo-gravimetry (adapted from Regulation (EC) No 152/2009)
Energy (kcal/100 g of NF)	402	409	404	403	406	/	/	Regulation (EU) 1169/2011

Abbreviations: /, not provided; AOAC, Association of Official Agricultural Chemists; EC, European Commission; EU, European Union; N, Nitrogen.

^aThe term is used as synonymous of non-digestible carbohydrates and does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence laid down in Annex I of Regulation (EC) 1169/2011.

The Panel notes that the crude protein content is listed in Tables 1–3, which incorporates non-protein nitrogen into the calculation, overestimates the true protein content in the NF, primarily due to the presence of chitin (Janssen et al., 2017). This point will be addressed in detail in Section 3.9 ‘Nutritional information’.

Chitin is the main form of non-digestible carbohydrates in *A. domesticus* (Hahn et al., 2018). Chitin is a linear polysaccharide constituted by β -(1,4)-linked 2-amino-2-deoxy- β -D-glucopyranose and 2-acetamido-2-deoxy- β -D-glucopyranose residues (Roberts, 1992). The applicant provided analytical data on the levels of chitin in five independently produced batches of AD frozen, AD dried and AD powder (Table 4).

The Panel notes that a nationally or internationally recognised reference method for the analytical determination of chitin 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 (Tables 1–3) and chitin (Table 4) could be due to the different analytical methods utilised. Additionally, the panel notes that some of the analytical results in Tables 1–4 do not concern the same NF batches.

TABLE 4 Chitin content of the NF.

NF form	Batch number									
	#2	#3	#4	#5	#6	#7	#8	#9		
AD frozen										
ADF (g/100 g) ^a	2.8	2.8	2.7	3.0	4.1	3.6	3.8	3.8		
ADL (Lignin) (g/100 g) ^b	<0.5	<0.5	0.5	0.6	0.8	0.6	0.8	0.7		
Chitin (g/100 g) ^c	2.8	2.8	2.2	2.4	3.3	3.0	3.0	3.1		
AD dried	Batch number									
	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20
ADF (g/100 g) ^a	11.5	11.4	10.3	10.4	10.1	9.8	9.3	10.3	8.3	9.4
ADL (Lignin) (g/100 g) ^b	2.1	2.1	1.8	1.7	1.7	1.6	1.4	1.4	1.5	1.7
Chitin (g/100 g) ^c	9.4	9.3	8.5	8.7	8.4	8.2	7.9	8.9	6.8	7.7
AD powder	Batch number									
	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30
ADF (g/100 g) ^a	14.7	13.7	15.5	5.4	15.7	8.0	9.4	9.8	8.8	8.8
ADL (Lignin) (g/100 g) ^b	2.9	2.6	3.4	1.1	3.0	1.5	1.3	1.4	1.6	1.5
Chitin (g/100 g) ^c	11.8	11.1	12.1	4.3	12.7	6.5	8.1	8.4	7.2	7.3

^aAcid detergent fibre (AFNOR NF V18-122, 1997).

^bAcid detergent lignin (AFNOR NF V18-122, 1997).

^cChitin calculated as ADF-ADL.

Concentrations of heavy metals in AD frozen, AD dried and AD powder are reported in Table 5.

The Panel notes that the concentrations of heavy metals reported for the NF are below maximum levels set for other foods, as well as to those previously reported and assessed for other foods derived from *A. domesticus* (EFSA NDA Panel, 2021, 2022) and that in the current EU legislation, no maximum levels of heavy metals are set for insects and products thereof as food.

TABLE 5 Heavy metal levels in AD frozen, AD dried, AD powder.

NF form	Unit	Batch number					Analytical method
		#2	#3	#4	#5	#9	
AD frozen							
Arsenic (As)	mg/kg	< 0.02	< 0.02	< 0.02	< 0.02	0.03	ICP-MS (digestion according to NEN-EN 13805)
Mercury (Hg)	mg/kg	< 0.01	< 0.01	< 0.01	< 0.01	0.012	
Lead (Pb)	mg/kg	0.033 ^a	0.038	0.034	0.035	0.033	
Cadmium (Cd)	mg/kg	0.013	0.011	0.015	0.014	0.012	
		Batch number					Analytical method
AD dried		#49	#50	#51	#52	#53	
Arsenic (As)	mg/kg	0.056	0.058	0.022	0.064	0.055	ICP-MS (according to NEN-EN 13805)
Mercury (Hg)	mg/kg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	
Lead (Pb)	mg/kg	0.053	0.054	< 0.01	0.063	0.058	
Cadmium (Cd)	mg/kg	0.035	0.028	0.091	0.029	0.03	
		Batch number					Analytical method
AD powder		#59	#60	#61	#62	#63	
Arsenic (As)	mg/kg	0.064	0.056	0.064	0.062	0.060	ICP-MS (according to NEN-EN 13805)
Mercury (Hg)	mg/kg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	
Lead (Pb)	mg/kg	0.072	0.075	0.074	0.070	0.068	
Cadmium (Cd)	mg/kg	0.030	0.027	0.026	0.026	0.025	

Abbreviations: ICP-MS, inductively coupled plasma - mass spectrometry; NEN-EN, Netherlands Standardization Institute - European Standard.

^aBatch #10.

Analytical data on levels of aflatoxins B1, B2, G1, G2, ochratoxin A, nivalenol, deoxynivalenol, zearalenone and fumonisins B1 and B2 in AD frozen, dried and powder were provided (Table 6). Values were lower than the maximum levels set for different foods in Regulation (EU) No 2023/915. The Panel notes that, in the current EU legislation, no maximum levels of mycotoxins are set for insects as food.

TABLE 6 Mycotoxins in the NF (AD frozen, AD dried, AD powder).

Mycotoxins (µg/kg)	Batches*					Analytical method
Aflatoxins B1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	Internal method, LC-MS/MS
Aflatoxins B2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins G1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins G2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins (sum of B1, B2, G1, G2)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	
Ochratoxin A	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	
Deoxynivalenol	< 20	< 20	< 20	< 20	< 20	
Zearalenone	< 10	< 10	< 10	< 10	< 10	
Fumonisin (sum of B1 + B2)	< 40	< 40	< 40	< 40	< 40	

Abbreviation: LC-MS/MS, Liquid chromatography with tandem mass spectrometry.

*Batch number 1, 2, 3, 4, 5 were analysed for AD dried; batch number 11, 12, 13, 14, 15 were analysed for AD frozen; batch number 21, 22, 23, 24, 25 were analysed for AD powder.

Analytical data on the pesticide concentrations of five independently produced batches of all forms of the NF have been provided. The results showed that the tested pesticide concentrations in the NF are below the limits of quantification (0.01 mg/kg) of the implemented methods (GC-MS/MS and LC-MS/MS) with the exception of piperonyl butoxide for which concentrations are between 0.012 and 0.020 mg/kg in AD dried, and 0.010–0.012 mg/kg in AD powder. The Panel notes that piperonyl butoxide is not subject to EU MRL legislation, as it is classified as a synergist rather than an active substance, and consequently, no EU MRLs have been established for this compound (EFSA, 2022).

Furthermore, the Panel notes that the Codex Alimentarius (Codex Alimentarius, 2024) has established maximum limits for piperonyl butoxide in various food commodities, ranging from 0.05 mg/kg in milks, 1 mg/kg in peanuts to 10 mg/kg in wheat flour and 7 mg/kg in poultry meat, all of which are substantially higher than the levels detected in the NF under consideration. Given the origin of the feed and the absence of prion or prion-related encoding genes in insects, no risk of developing prion diseases is associated with the consumption of the NF (EFSA Scientific Committee, 2015).

After EFSA's request, the applicant provided analytical data on the levels of various organic contaminants present in the five batches of AD powder (Table 7). All values were below regulatory limits established for other food matrices.

TABLE 7 Levels of organic contaminants in the NF (AD powder).

Parameter	Unit	Batch number					Analytical method
		#59	#60	#61	#62	#63	
2-MCPD (free form)	µg/kg	<5	<5	<5	<5	<5	Internal method, GC–MS/MS
3-MCPD (free form)	µg/kg	<5	<5	<5	<5	<5	Internal method, GC–MS/MS
PFAS ^a	µg/kg	<0.4	<0.4	<0.4	<0.4	<0.4	Internal method, LC–MS/MS
Sum of PAHs ^b	µg/kg	<2.6	<2.0	<2.0	<2.0	<2.0	Internal method, APGC–MS/MS
Furans	µg/kg	<5	<5	<5	<5	<5	US FDA/CFSAN 2006–10
Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ) ^c	pg/g fat	0.64	0.69	0.67	0.66	0.66	Conform Regulation (EU) 2017/644 (food) and Regulation (EU) 2017/771 (feed)

Abbreviations: 2-MCPD & 3-MCPD, 2-Mono-chloropropane-diol & 3-mono-chloropropane-diol; APGC-MS/MS, Atmospheric Pressure Gas Chromatography Tandem Mass Spectrometry; EU, European Union; GC-MS/MS, Gas Chromatography with Tandem Mass Spectrometry; LC–MS/MS, Liquid Chromatography with Tandem Mass Spectrometry; WHO, World Health Organization; US FDA/CFSAN, U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition.

^aPFAS: Per- and polyfluoroalkyl substances (including perfluorooctane sulfonic acid, perfluorooctanoic acid, perfluorononanoic acid, perfluorhexanesulfonic acid).

^bPAHs: polycyclic aromatic hydrocarbons (including benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, chrysene).

^cSum of polychlorinated dibenzo-*p*-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls.

The applicant provided microbiological data on five independently produced batches of the NF forms AD frozen (Table 8), AD dried (Table 9) and AD powder (Table 10). The Panel notes that the microbiological values of the analysed samples do not exceed the specification limits defined in Section 3.5.

TABLE 8 Batch-to-batch microbiological analyses of the NF form AD frozen.

NF form	Unit	Batch number					Analytical method
		#6	#7	#8	#9	#10	
Total aerobic count	cfu/g	300	210	130	200	300	ISO 4833-1:2013
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 16649-2:2001
<i>Listeria monocytogenes</i>	in 25g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 7932:2004
Coagulase positive staphylococci	cfu/g	< 10 ^{#,a}	< 10 ^{#,b}	< 10 ^{#,c}	< 10 ^{#,d}	< 10 ^{#,e}	ISO 6888-2:2021
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 7937:2004
Sulfite-reducing bacteria	cfu/g	/	< 10 ^{#b}	< 10 ^{#c}	< 10 ^{#d}	< 10 ^{#e}	ISO 15213:2003
Yeast & moulds	cfu/g	< 100 ^{#,a}	< 100	< 100	< 100	< 100	ISO 21527-2:2008

Abbreviations: /, not provided; cfu, colony forming units; EN, European Norm; ISO, International Organization for Standardization; NF, Norme Française.

^{#a,b,c,d,e}The batch identification number is, respectively, 1, 2, 3, 4, 5.

TABLE 9 Batch-to-batch microbiological analyses of the NF form AD dried.

NF form	Unit	Batch number					Analytical method
		#11	#13	#14	#15	#49	
AD frozen							
Total aerobic count	cfu/g	40	<40	<40	70	1300	ISO 4833-1:2013
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 16649-2:2001
<i>Listeria monocytogenes</i>	in 25g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 7932:2004
Coagulase positive staphylococci	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 6888-2:2021
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 7937:2004
Sulfite-reducing bacteria	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 15213:2003
Yeast & moulds	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 21527-2:2008

Abbreviations: /, not provided; cfu, colony forming units; EN, European Norm; ISO, International Organization for Standardization; NF, Norme Française.

TABLE 10 Batch-to-batch microbiological analyses of the NF form AD powder.

NF form	Batch number						Analytical method
	Unit	#26	#27	#28	#29	#30	
AD frozen							
Total aerobic count	cfu/g	3500	14,000	18,000	1300	8400	ISO 4833-1:2013
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 16649-2:2001
<i>Listeria monocytogenes</i>	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 7932:2004
Coagulase positive staphylococci	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 6888-2:2021
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 7937:2004
Sulfite-reducing bacteria	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 15213:2003
Yeast & moulds	cfu/g	< 100	< 100	< 100	< 100	100–400	ISO 21527-2:2008

Abbreviations: /, not provided; cfu, colony forming units; EN, European Norm; ISO, International Organization for Standardization; NF, Norme Française.

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

3.4.1 | Stability

The applicant performed stability tests with five independently produced batches of the NF forms AD frozen (Table 11), AD dried (Table 12) and AD powder (Table 13). The NF form AD frozen is to be stored at -18°C , in closed packages, with an intended shelf-life of 10 months. AD dried and AD powder are to be stored at room temperature, in closed packages, with an intended shelf-life of 10 months. The stability tests were carried out storing the dried and powder forms at room temperature, and the frozen form at -18°C for 10 months.

TABLE 11 Microbiological stability of AD frozen under standard storage conditions.

NF form	Batch number						Analytical method
	Unit	#44	#45	#46	#47	#48	
Total aerobic count	cfu/g	70	< 40	< 40	80	60	ISO 4833-1:2013
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 16649-2:2001
<i>Listeria monocytogenes</i>	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 79-32:2004
Coagulase positive staphylococci	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 6888-2:2021
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 79-37:2004
Sulfite-reducing bacteria	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 15213:2003
Yeast & moulds	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 21527-2:2008

Abbreviations: cfu, colony forming units; EN, European Norm; ISO, International Organization for Standardization; NF, Norme Française.

TABLE 12 Microbiological stability of AD dried under standard storage conditions.

NF form	Batch number						Analytical method
	Unit	#54	#55	#56	#57	#58	
Total aerobic count	cfu/g	340	< 40	380	70	770	ISO 4833-1:2013
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 16649-2:2001
<i>Listeria monocytogenes</i>	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 79-32:2004
Coagulase positive staphylococci	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 6888-2:2021
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 79-37:2004
Sulfite-reducing bacteria	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 15213:2003
Yeast & moulds	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 21527-2:2008

Abbreviations: cfu, colony forming units; EN, European Norm; ISO, International Organization for Standardization; NF, Norme Française.

TABLE 13 Microbiological stability of AD powder under standard storage conditions.

NF form	Batch number						Analytical method
	Unit	#54	#55	#56	#57	#58	
Total aerobic count	cfu/g	190	210	120	2100	90	ISO 4833-1:2013
<i>Enterobacteriaceae</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 21528-2:2017
<i>Escherichia coli</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	NF ISO 16649-2:2001
<i>Listeria monocytogenes</i>	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 11290-1:2017
<i>Salmonella</i> spp.	in 25 g	Not detected	Not detected	Not detected	Not detected	Not detected	NF EN ISO 6579-1:2017
<i>Bacillus cereus</i>	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 79-32:2004
Coagulase-positive staphylococci	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 6888-2:2021
<i>Clostridium perfringens</i>	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 79-37:2004
Sulfite-reducing bacteria	cfu/g	< 10	< 10	< 10	< 10	< 10	ISO 15213:2003
Yeast & moulds	cfu/g	< 100	< 100	< 100	< 100	< 100	ISO 21527-2:2008

Abbreviations: cfu, colony forming units; EN, European Norm; ISO, International Organization for Standardization; NF, Norme Française.

Furthermore, the applicant provided analytical data on the water activity, oxidative status of the lipids of all forms of the NF, at time 0 and 10 months, under the standard storage conditions (Table 14).

The Panel notes that the samples analysed at $t=0$ were not always the same NF batches analysed at later time points. Nevertheless, the Panel notes that the values of none of the analysed batches exceeded the given specification limits.

The Panel notes that the water activity values in the NF forms AD dried and AD powder at the beginning and at the end of the shelf-life are stable and low, acting as a deterrent to the microbiological growth in the NF forms.

The applicant provided analytical data for histamine for at least four independently produced batches of all forms of the NF at $t=0$ and $t=10$ months (end of shelf-life). Histamine levels in the NF at time zero of the shelf-life ranged between 1.07 and 2.36 mg/kg in AD frozen, 2.74 and 3.36 mg/kg in AD dried, 1.11 and 2.6 mg/kg in AD powder (method Czech Journal of Food Science, Vol, 21- No. 5: 167–175 LC-UV/DAD). Levels of histamine in the NF measured at the end of the shelf-life ranged between 1.12 and 2.18 mg/kg in AD dried and 3.61 and 4.50 mg/kg in AD powder. The histamine values are much lower compared to the limit of 200 mg/kg for histamine in fishery products set in Regulation (EC) No 2073/2005.⁵

The Panel notes that the free fatty acid concentration in the powdered form has increased markedly after 10 months of storage. The Panel considers that no safety concerns arise from this increase.

⁵Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

TABLE 14 Oxidative stability and water activity of AD frozen, AD dried, AD powder.

NF form	Storage time (months)					Storage time (months)					Analytical method
	0	0	0	0	0	10	10	10	10	10	
AD frozen											
Water activity	0.98	0.98	0.99	0.98	0.99	0.99	0.99	0.99	0.99	0.98	Hygrometry (dew point)
Free fatty acids (% of fat)	7.47	5.86	6.46	6.00	6.73	11.2	4.06	3.73	n.d.	5.28	ISO 666: 2009
Peroxide (meq O ₂ /kg fat)	2.5	3.3	1.9	1.9	1.5	3.2	2.0	3.7	4.1	2.8	ISO 27140: 2009
<i>p</i> -Anisidine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.31	5.20	n.d.	7.5	AOCS Cd 18–90 (2017)
AD dried											
Water activity	0.57	0.58	0.57	0.57	0.50	0.50	0.54	0.50	0.51	0.50	Hygrometry (dew point)
Free fatty acids (% of fat)	6.84	7.85	7.57	7.79	6.52	7.93	8.69	8.07	12.31	8.39	ISO 666: 2009
Peroxide (meq O ₂ /kg fat)	16.00	6.3	4.6	12.1	<30	3.3	2.9	2.9	3.1	3	ISO 27140: 2009
<i>p</i> -Anisidine	n.d.	n.d.	n.d.	n.d.	n.d.	3.42	1.3	1.34	1.41	1.45	AOCS Cd 18–90 (2017)
AD powder											
Water activity	0.43	0.43	0.32	0.35	0.31	0.35	0.36	0.35	0.36	0.38	Hygrometry (dew point)
Free fatty acids (% of fat)	7.66	8.91	8.90	8.03	10.14	41.84	40.39	42.24	41.38	41.56	ISO 666: 2009
Peroxide (meq O ₂ /kg fat)	4.50	5.30	3.00	6.60	6.70	2.4	2.4	2.5	2.6	2.4	64 LFGB L 13.00–40:2012–01
<i>p</i> -Anisidine	n.d.	n.d.	n.d.	n.d.	n.d.	1.78	1.93	1.78	1.85	1.68	AOCS Cd 18–90 (2017)

Abbreviations: AOCS, American Oil Chemists' Society; ISO, International Organization for Standardization; LFGB, Lebensmittel- und Futtermittelgesetzbuch; n.d., not determined.

Stability in the intended for use matrices

Given the intended use of the NF as an ingredient in various food products, EFSA requested that the applicant investigate its stability in composite foods.

Cereal bars with the NF comprised 5% of AD powder and a mixture of other ingredients (chicory root fibre, dark chocolate, soy protein, rice protein, grated coconut, cocoa butter, soy protein extrudates, flavourings). Cereal bars were baked for 10 min at 180°C and stored at room temperature in sealed packages. Cereal bars without the NF were analysed as control sample.

The applicant reported the microbiological content, processing contaminants and oxidative status of cereal bars with AD powder after manufacturing (Table 15). The applicant investigated one sample per composite food.

TABLE 15 Stability of the NF in the matrices intended for use, at time of production.

Parameter	Units	Cereal bars with NF	Cereal bars without NF	Analytical method
Fat oxidation				
Free fatty acids	% of fat	2.21	1.94	ISO 666: 2009
Peroxide	meq O ₂ /kg fat	1.1	<0.4	ISO 27140: 2009
Processing contaminants				
Furan	µg/kg	<5	<5	US FDA/CFSAN 2006–10
Acrylamide	µg/kg	<30	<30	Internal LC–MS/MS
PAHs	µg/kg	<2.0	<2.2	Internal APGC-MS/MS
Histamine	mg/kg	<1	<1	LC-UV/DAD (Czech J Food Sci, Vol 21)
Microbiology				
Total aerobic count	cfu/g	270	5700	ISO 4833-1
Heat-resistant mesophilic spore count	cfu/g	<10	<10	Internal- E-cultural technique
<i>Bacillus cereus</i>	cfu/g	<100	<100	ISO 7932:2004
<i>Clostridium perfringens</i>	cfu/g	<10	<10	ISO 7937:2004

Abbreviations: APGC-MS/MS, Atmospheric Pressure Gas Chromatography Tandem Mass Spectrometry; CFSAN, Center for Food Safety and Applied Nutrition; LC–MS/MS, Liquid Chromatography–Tandem Mass Spectrometry; ISO, International Organization for Standardization; PAHs, polycyclic aromatic hydrocarbons (including benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, chrysene); US FDA, United States Food and Drug Administration.

The Panel notes that the analytical data regarding the putative formation of contaminants, lipid oxidation and microbiological content on the use of NF as an ingredient in cereal bars are limited. The Panel further notes that the food items containing the NF have to comply with existing legislative limits, such as microbiological levels set in Regulation (EC) No 2073/2005 and the benchmark levels of acrylamide in bakery products established by Regulation (EU) 2017/2158.⁶

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

3.5 | Specifications

The specifications of the NF are indicated in Table 16.

⁶Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food.

TABLE 16 Specifications of the NF.

Description:				
AD frozen: whole, blanched and frozen <i>A. domesticus</i> adult				
AD dried, whole, blanched, dried <i>A. domesticus</i> adult				
AD powder: whole, blanched, dried and ground <i>A. domesticus</i> adult				
Parameters	Units	AD frozen	AD dried	AD powder
Appearance		Grey-brown colour, 16–21 mm length	Grey-brown colour, 16–21 mm length	Grey-brown colour, powder
Moisture	g/100 g	65–80	2–10	2–10
Crude protein (N×6.25)	g/100 g	15–25	45–65	50–70
Fat	g/100 g	3.5–9.0	12–25	10–18
– of which saturated		– 1.0–3.0	– 4.0–10.0	3.0–5.0
Carbohydrates	g/100 g	0.1–2.5	0.5–10	0.1–5.0
Dietary fibre	g/100 g	1.0–2.5	5.0–10.0	5.0–10.0
Chitin	g/100 g	1.5–5.0	5.5–10.0	4.0–12
Ash	g/100 g	1.0–2.0	4.0–6.0	4.0–6.0
Peroxide value	meq O ₂ /kg fat	<5	<5	<5
Heavy metals				
Cadmium (Cd)	µg/kg	<20	<100	<100
Lead (Pb)	µg/kg	<50	<75	<75
Arsenic (As)	µg/kg	<30	<70	<70
Mercury (Hg)	µg/kg	<25	<25	<25
Minerals				
Manganese	mg/kg	<50	<100	<100
Mycotoxins				
Total aflatoxin (sum of B1, B2, G1, G2)	µg/kg	<0.4	<0.4	<0.4
Ochratoxin A	µg/kg	<0.2	<0.2	<0.2
Deoxynivalenol	µg/kg	<20	<20	<20
Zearalenone	µg/kg	<10	<10	<10
Fumonisin (sum of B1, B2)	µg/kg	<40	<40	<40
Microbiological				
TAMC	cfu/g	<10 ^{AS}	<10 ^{AS}	<10 ^{AS}
<i>Enterobacteriaceae</i>	cfu/g	<10	<10	<10
<i>Escherichia coli</i>	cfu/g	<10	<10	<10
<i>Listeria monocytogenes</i>	in 25 g	Not detected	Not detected	Not detected
<i>Salmonella</i>	in 25 g	Not detected	Not detected	Not detected
Presumptive <i>Bacillus cereus</i>	cfu/g	<100	<100	<100
<i>Clostridium perfringens</i>	cfu/g	<10	<10	<10
Sulfite-reducing anaerobes	cfu/g	<10	<10	<10
Yeast and Moulds	cfu/g	<100	<100	<100
Other contaminants				
Sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ)	pg/g fat	<1.25	<1.25	<1.25

Abbreviations: cfu, colony forming units; N, Nitrogen; PCDD/F-PCB-TEQ, sum of polychlorinated dibenzo-*p*-dioxins-polychlorinated dibenzofurans-polychlorinated biphenyls; TAMC, total aerobic microbial count; WHO, World Health Organization.

The Panel notes that the content of peroxide value in some batches of the NF deviate from the limit set in the specifications, and considers a limit of 5 meq O₂/kg fat for peroxide value to be appropriate for the NF.

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

According to the information provided by the applicant, the NF has been commercially available in some EU countries by ALBinsecta for a few years, primarily in the form of dried product, with an estimated annual production volume of a few tonnes.

A. domesticus either collected from the wild or reared in farms is consumed as part of the customary diet in some non-EU countries. Their consumption by humans has been reported mainly in Thailand (Hanboonsong et al., 2013; Yen, 2015) and Lao PDR (Codex Alimentarius Commission, 2010; Durst & Hanboonsong, 2015), but also Cambodia (FAO, 2013), Ghana (Anankware et al., 2016), Mexico (Ramos-Elorduy, 2009), Democratic Republic of Congo and Kenya (Halloran et al., 2018).

Hanboonsong et al. (2013) reported that around 20,000 *A. domesticus* small- and medium-sized farms are registered in Thailand. Products are distributed to wholesalers and local markets. Commercial chicken feed and vegetables are used as substrate and 7500 tons of crickets (including *A. domesticus*) a year are produced. In 2017, the Thai Agricultural Standards Committee established Good Agricultural Farming Practices for cricket farming including *A. domesticus* (ACFS, 2017).

A. domesticus is also farmed in Lao PDR (Hanboonsong & Durst, 2014), as well as, to a lesser extent, in Cambodia, the Democratic Republic of Congo and Kenya (Halloran et al., 2018).

Additionally, in Australia and New Zealand, it is considered as not NFstuff and no safety concerns were identified with the exception of potential risk of allergenicity in crustacean-allergic or other sensitive individuals when consuming crickets or foods derived from crickets (FSANZ, 2020). Since 1 May 2017, *A. domesticus* in adult phase is among the insect species that can be legally introduced in the Swiss market as food (whole, chopped or ground). In Canada, it is considered non-novel for use as a food or food ingredient (Health Canada, 2021). *A. domesticus* is marketed for human consumption in the EU, Australia and USA as a whole insect or as a food ingredient in several food products (e.g. nutritional bars, lollipops, flour, chocolate, etc.).

In the EU and based on EFSA opinions adopted in July 2021 and March 2022, the Commission adopted Commission Implementing Regulation (EU) 2022/188⁷ authorising the placing on the market of frozen, dried and powder forms of *A. domesticus* and Commission Implementing Regulation (EU) 2023/5⁸ authorising the placing on the market of partially defatted powder of *A. domesticus* (house cricket).

3.7 | Proposed uses and use levels and anticipated intake

3.7.1 | Target population

The target population proposed by the applicant is the general population.

3.7.2 | Proposed uses and use levels

The applicant proposed to use the NF as an ingredient in several food products. These food products are defined using the FoodEx2 hierarchy, and the maximum use levels are reported in Table 17. The applicant intends to use different forms of the NF (frozen, dried, powder) separately in the respective food categories, and not in combination.

TABLE 17 Food categories and maximum use levels intended by the applicant.

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)		
			AD frozen	AD dried	AD powder
3	A005Y	Crackers and breadsticks	9.6	4.8	4.8
4	A00EZ	Cereal bars plain	17.8	8.9	8.9
4	A00FA	Cereal bars mixed	17.8	8.9	8.9
5	A034G	Bitter chocolate	2.0	1.0	1.0
5	A034S	Pralines	2.0	1.0	1.0
5	A034R	Chocolate-coated confectionery	2.0	1.0	1.0
5	A0C6P	Chocolate spread	2.0	1.0	1.0
4	A040C	Finger food	9.6	4.8	4.8
5	A041D	Paella	10.0	5.0	5.0

(Continues)

⁷Commission Implementing Regulation (EU) 2022/188 of 10 February 2022 authorising the placing on the market of frozen, dried and powder forms of *Acheta domesticus* 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.

⁸Commission Implementing Regulation (EU) 2023/5 of 3 January 2023 authorising the placing on the market of *Acheta domesticus* (house cricket) partially defatted powder as a novel food and amending Implementing Regulation (EU) 2017/2470.

TABLE 17 (Continued)

FoodEx2 level	FoodEx2 code	Food category	Max use level (g NF/100 g)		
			AD frozen	AD dried	AD powder
5	A041C	Nasi goreng	10.0	5.0	5.0
5	A041F	Risotto	10.0	5.0	5.0
5	A041G	Rice and vegetables meal	10.0	5.0	5.0
5	A041J	Rice, meat and vegetables meal	10.0	5.0	5.0
4	A042E	Caesar salad	10.0	5.0	5.0
4	A042F	Greek salad	10.0	5.0	5.0
4	A042G	Prepared legume (beans) salad	10.0	5.0	5.0

3.7.3 | Anticipated intake of the NF

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 17), using the EFSA Dietary Exposure (DietEx) Tool,⁹ which is based on individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest means and 95th percentiles (P95) anticipated daily intakes of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Tables 18 and 19.

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).

TABLE 18 Intake estimate (mg/kg bw per day) 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	0.1	2.6	0.0	20.0
Young children ^c	1 to < 3	0.6	22.2	0.0	109.1
Other children	3 to < 10	0.2	20.4	0.5	91.2
Adolescents	10 to < 18	0.7	16.4	2.0	72.6
Adults ^d	≥ 18	0.6	9.8	1.3	51.4

Abbreviations: bw, body weight; P95, 95th percentile.

^aIntakes are assessed for all EU dietary surveys available in the food comprehensive database on 28/11/2023. The lowest and the highest averages observed among all EU surveys are reported in these columns.

^bIntakes are assessed for all EU dietary surveys available in the food comprehensive database on 28/11/2023. 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).

^cReferred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

^dIncludes elderly, very elderly, pregnant and lactating women.

TABLE 19 Intake estimate (mg per day) 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 per day)		P95 intake (mg per day)	
		Lowest ^a	Highest ^a	Lowest ^b	Highest ^b
Infants	< 1	0.6	23.4	0.0	176.0
Young children ^c	1 to < 3	5.8	269.9	0.0	1271.0
Other children	3 to < 10	3.2	458.9	10.3	2101.2
Adolescents	10 to < 18	35.1	859.1	110.0	3600.0
Adults ^d	≥ 18	38.8	761.3	80.0	4006.6

Abbreviation: P95, 95th percentile.

^aIntakes are assessed for all EU dietary surveys available in the food comprehensive database on 19/08/2024. The lowest and the highest averages observed among all EU surveys are reported in these columns.

^bIntakes are assessed for all EU dietary surveys available in the food comprehensive database on 19/08/2024. 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).

^cReferred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

^dIncludes elderly, very elderly, pregnant and lactating women.

⁹<https://www.efsa.europa.eu/it/science/tools-and-resources/dietex>.

3.7.4 | Estimate of exposure to undesirable substances

Based on the P95 intake estimates (Tables 18 and 19), EFSA calculated the exposure to undesirable substances (heavy metals, mycotoxins and organic contaminants, antinutritional factors), for all population groups. The specification limits were used as maximum values for the concentration of substances considered. When specification limits for a substance have not been proposed, the maximum values reported for the analysed batches were used.

The Panel considers that the consumption of the NF under the proposed uses and use levels does not contribute substantially to the overall intake of undesirable substances through diet.

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

No ADME data are required for the NF.

3.9 | Nutritional information

The applicant provided nutritional analysis of the NF. The NF consists mainly of protein, fat, digestible carbohydrates, dietary fibre (mainly chitin) and inorganic matter. Due to the higher water content in the undried form compared to the dried forms, the concentrations of these components are lower in AD frozen compared to AD dried and AD powder. Analytical data on the amino acid composition (Appendix A), the fatty acid composition (Appendix B), minerals and vitamins have been provided for several batches of the NF forms.

3.9.1 | Protein content and protein quality

For regulatory purposes with respect to nutritional labelling, protein content 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 1169/2011¹⁰ on the provision of food information to consumers). Using this factor, the NF contains an average of 20.6 g of crude protein per 100 g of AD frozen, 63.1 g of crude protein per 100 g of AD dried and 68.1 g of crude protein per 100 g of AD powder.

The applicant quantified the content of amino acids in five batches of each form of the NF according to ISO13903:2005 and Commission Regulation (EC) No 152/2009¹¹ (Appendix A), and all essential amino acids were found to be present.

The applicant investigated the in-vitro pepsin digestibility of all three NF forms using the method ISO 6655:1997. The method is based on the amount of nitrogen and does not distinguish between nitrogen from protein and other sources. Based on crude protein, the resulting in-vitro digestibility was 72.7% for AD frozen (range 70.5%–74.9%), 80% for AD dried (range 78.9%–80.1%) and 77.3% for AD powder (range 77.1%–77.6%).

The Panel notes that the use of the conventional nitrogen conversion factor overestimates the true protein content of the NF due to the presence of non-protein nitrogen derived mainly from chitin (Janssen et al., 2017).

Boulos et al. (2020) determined an average nitrogen conversion factor of 5.25 for whole *A. domesticus* as supported by literature data. Based on the amino acid profile of the NF and following a methodological approach presented by Biancarosa et al. (2017), the applicant determined average conversion factors of 4.88, 4.61 and 4.09 for AD frozen, AD dried and AD powder, respectively. The average conversion factor for all forms of the NF was 4.53. Using this factor, the protein content of the NF forms ranges from 14.3 to 15.2 g/100 g in AD frozen, from 44.4 to 46.4 g/100 g in AD dried and from 48.6 to 49.7 g/100 g in AD powder.

The applicant provided data on the amino acid content to the crude and true protein content for the three forms of the NF, where true protein is defined by using the newly defined conversion factors for each form of the NF (Appendix A). The Panel notes that, compared to the recommended amino acid scoring pattern for infants (FAO, 2013), tryptophan is the first limiting amino acid in the true protein of all three forms of the NF.

Regarding the age groups children '6 months to 3 years' and 'older child, adolescent, adult', the essential amino acids of the protein in all three forms of the NF meet the values given in the respective recommended amino acid scoring patterns by FAO (2013).

In an attempt to estimate protein quality of the NF, as recommended by FAO (2013), the applicant calculated the digestible indispensable amino acid scores (DIAAS) based on crude protein contents for all three forms. Resulting DIAAS¹² values for 'infants', 'child 6 months to 3 years' and 'older child, adolescent, adult', respectively, for AD frozen were 57%, 79% and 93%; for AD dried 58%, 82% and 97%; and for AD powder 53%, 72% and 86%. The Panel notes that measurements of

¹⁰Regulation (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.

¹¹Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed.

¹²Values of DIAAS were obtained as product of the lowest amino acid content in the true protein of the NF in comparison to the recommended amino acid scoring pattern (FAO, 2013), and pepsin digestibility of the crude protein.

digestibility of individual amino acids of the NF forms were not available, and therefore, the respective values for pepsin digestibility of the crude protein were used in the DIAAS calculations. The Panel notes that the use of crude protein in the DIAAS calculations overestimates the amount of protein, leading consequently to a lower DIAAS value.

The Panel notes that the consumption of the NF at the proposed uses and use levels is not expected to negatively impact protein nutrition of the EU population.

3.9.2 | Fatty acids, vitamins and minerals

The major fatty acids in all forms of the NF are linoleic acid, oleic acid and stearic acid (Appendix B). On average among the forms of the NF, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids constitute, respectively, $42.55 \pm 6.69\%$, $23.83 \pm 1.96\%$ and $32.92 \pm 5.87\%$ of the total fatty acids (gas chromatography with flame-ionisation detection). The average *trans*-fatty acid content among all NFs is $0.56 \pm 0.11\%$ of total fatty acids.

The applicant provided analytical data on the levels of some minerals and vitamins (Appendices C and D).

Considering the concentrations reported in the appendices, and the estimated P95 of exposure of the NF (Tables 18 and 19), the Panel notes that none of the existing upper levels for the analysed micronutrients are expected to be exceeded, for any population groups.

The NDA Panel estimated the intake of Manganese from the NF considering the product specification for Mn and the estimated daily intake of the NF for all population groups (Tables 18 and 19). The highest estimated P95 intake of Mn (considering the highest Mn value in the NF of 100 mg/kg) from the NF is 0.017 mg/day in infants, 0.13 mg/day in toddlers, 0.21 mg/day in other children, 0.36 mg/day in adolescents and 0.40 mg/day in adults.

The Panel considers that such intake of Mn (<5% of the highest background dietary intake¹³) from the NF is not of concern.

3.9.3 | Antinutritional factors

Insects may contain antinutritional factors such as tannins, oxalates, phytates, hydrogen cyanide (Meyer-Rochow et al., 2021; Shantibala et al., 2014), thiaminases (Nishimune et al., 2000) and protease inhibitors (Eguchi, 1993). The applicant determined the concentration of total polyphenols (Table 20), phytic acid, oxalic acid, calcium oxalate, trypsin inhibitor activity and total hydrogen cyanide in five batches of the NF form AD powder (Table 21). The reported values in the NF do not exceed the occurrence levels of these compounds in other foodstuffs (EFSA CONTAM Panel, 2019; Gupta, 1987; Holmes & Kennedy, 2000; Rao & Prabhavathi, 1982; Schlemmer et al., 2009) and other insects (EFSA NDA Panel, 2021, 2022, 2024).

It has been reported that chitin can be partially digested in the human stomach by the acidic mammalian chitinase (AMCase) (Muzzarelli et al., 2012; Paoletti et al., 2009). The NF contains on average 2.83 ± 0.36 g of chitin in 100 g of AD frozen, and 8.95 ± 2.8 g of chitin in 100 g of AD powder (Table 4). Nevertheless, 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. 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 (Anastopoulos et al., 2017; Franco et al., 2004) possibly negatively affecting their bioavailability, as reported for dietary fibres in general (Baye et al., 2017).

TABLE 20 Polyphenol content in the NF (AD powder).

Antinutrient	Unit	Batch number					
		#59	#60	#61	#62	#63	
Total polyphenols (gallic acid equivalent)	mg/kg	6930	6470	6470	6620	6440	Internal method, Spectrophotometry

¹³As reported by the published minutes of the 154th meeting of the working group on novel foods (WG NF 2024), the WG considers that 'for the purpose of the assessment of NFs, intakes that lead to a significant increase of manganese (Mn) intake as compared to the safe levels set by the EFSA NDA Panel (2023) are considered of concern. In the absence of adequate data to establish an UL, the NDA Panel (2023) established the safe levels of intake of Mn based on observed background intake of Mn among high consumers from the general population (P95 estimates). 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 from the NF exceeding 5% of the highest P95 background dietary intake is considered as a significant contribution.

TABLE 21 Antinutritional factors in the NF (AD powder).

Antinutrient	Unit	Batch number					
		#59	#60	#61	#62	#63	
Phytic acid	g/100 g	<0.14	<0.14	<0.14	<0.14	<0.14	Analytical Biochemistry Vol. 77:536–539 (1977)
Oxalic acid	g/100 g	0.02	0.03	0.03	0.03	0.03	Internal, IC-EC
Calcium oxalate	g/100 g	0.04	0.04	0.04	0.04	0.04	Internal, IC-EC
Trypsin inhibitor	mg/g	<0.2	<0.2	<0.2	<0.2	<0.2	NEN-EN-ISO 14902:2001
Hydrogen cyanide	mg/kg	<1	<1	<1	<1	<1	Internal method, HS-GC-NPD

Abbreviations: EN, European Norm; IC-EC, Ion Chromatography with Electrochemical Detection; ISO, International Organisation for Standardisation; HS-GC-NPD, head-space gas chromatography with nitrogen-phosphorus detection; NEN, Royal Netherlands Standardisation Institute.

3.10 | Toxicological information

No toxicological studies were provided with the NF under assessment. The applicant performed a literature search and no papers were found concerning the toxicology of *A. domesticus*.

The toxicological profile of *A. domesticus* has been previously assessed by the Panel (EFSA NDA Panel, 2021, 2022, 2024). The Panel concluded that no safety concerns arose from the history of use and compositional data of *A. domesticus*.

The Panel notes that the NF under assessment can be considered equivalent to previously assessed *A. domesticus* (EFSA NDA Panel, 2021, 2022, 2024). However, differences in feeding regimes and processing may result in different toxicological profiles. The Panel identified and assessed a recently published toxicological study available in literature (in vitro and in vivo genotoxicity, 14- and 90-day subchronic toxicity study) with processed (dried and sterilised) *A. domesticus* powder as the testing material (Yasuki et al., 2022). All tests were reported to follow relevant OECD guidelines.

In vitro genotoxicity was examined using a mammalian chromosomal aberration test on Chinese hamster lung (CHL-IU) cells, and no genotoxic effects were observed at concentrations up to 5000 µg/mL with and without metabolic activation (S9 mix).

In vivo genotoxicity was assessed using the micronucleus test in mice. The mice were administered 0, 500, 1000 and 2000 mg/kg bw of AD powder orally for two consecutive days. Cyclophosphamide monohydrate was used as a positive control. Bone marrow cells harvested 24 h post-treatment showed no increase in micronucleated polychromatic erythrocytes up to the highest tested dose.

Toxicity was evaluated over 14-day and 90-day periods in mice. The mice received 300, 1000 and 3000 mg/kg bw *A. domesticus* in both studies. The evaluation included haematological analysis, blood biochemistry (limited parameters in the 90-day study) and relative organ weight measurements. Mice were also observed for any signs of toxicity or behavioural changes. No histopathological investigation was performed.

There were no relevant differences in body weight gain, blood biochemistry and haematological tests in the groups that received 300, 1000 and 3000 mg/kg *A. domesticus* powder compared with the control group for both males and females. Similarly, organ weights showed no relevant deviations from control weights.

The Panel concludes that since these studies were not performed with the NF, the outcomes can only be considered as supporting evidence for the safety of the NF.

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) after intranasal or intraperitoneal administration (Komi et al., 2018). The Panel notes that no indications of immunotoxicity were identified in the 90-day toxicity study.

Considering the history of consumption of *A. domesticus*, the toxicological studies, the compositional information and the production process, the Panel considers that there is no concern regarding the toxicity of the NF.

3.11 | Allergenicity

The Panel has previously considered that the consumption of *A. domesticus* might induce primary sensitisation to *A. domesticus* proteins. The Panel also considered that allergic reactions may occur in subjects allergic to crustaceans, mites and molluscs (cross-reactivity) (EFSA NDA Panel, 2021, 2022, 2024).

From literature research, the Panel has noted that additional allergens may be found in the NF, if these allergens are present in the substrate fed to the insects. This may include allergens listed in Annex II of Regulation (EU) No 1169/2011.

4 | DISCUSSION

The NF which is subject of the application is house cricket (*Acheta domesticus*), in frozen, dried and powder form. The production process is sufficiently described and does not raise safety concerns. The Panel considers that the NF is sufficiently characterised. The NF consists mainly of protein, fat, dietary fibre (mainly chitin) and inorganic matter. The concentration of

contaminants in the NF may depend on their occurrence in the insect feed. Provided that applicable EU legislation regarding feed is followed, the consumption of the NF does not raise safety concerns.

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 forms as ingredients in several food products. The target population is the general population. The highest intake estimate per kg bw (i.e. 22 mg/kg bw per day) was calculated for the NF form AD dried for young children (1–<3 years old), corresponding to an estimated intake of 270 mg of the NF 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 is not expected to negatively impact protein nutrition and also does not contribute substantially to the total dietary exposure of analysed undesirable substances.

None of the existing upper levels of the analysed micronutrients are expected to be exceeded considering the proposed uses and use levels.

The reported concentrations of the antinutritional factors in the NF are similar to those in other foods. The Panel considers that chitin is not expected to be digested in the small intestine of humans to any significant degree and is assumed to be excreted mainly unchanged. 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. No safety concerns arise from the history of use and toxicological information of *A. domesticus*, or from the compositional data of the NF. The Panel considers that the consumption of the NF might trigger primary sensitisation to *A. domesticus* proteins. The Panel also considers that allergic reactions may occur in subjects allergic to crustaceans, mites and molluscs (cross-reactivity). Additionally, the Panel notes that allergens from the feed (e.g. gluten) may be present 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 may occur upon consumption.

6 | RECOMMENDATION

The Panel recommends that research should be undertaken on the allergenicity to *A. domesticus*, including cross-reactivity to other allergens.

7 | STEPS TAKEN BY EFSA

1. On 09/12/2022 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of frozen and dried formulations from whole house crickets (*Acheta domesticus*) as a novel food Ref. Ares(2022)8565035.
2. On 24/03/2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
3. On 24/10/2023, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
4. On 22/12/2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
5. On 27/07/2024, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
6. On 10/10/2024, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
7. On 14/10/2024, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
8. During its meeting on 30/10/2024, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of frozen, dried and powder forms of house crickets (*Acheta domesticus*) as a NF pursuant to Regulation (EU) 2015/2283.

GLOSSARY AND/OR ABBREVIATIONS

A.	<i>Acheta</i>
AAA	Aromatic amino acids
ACFS	Thailand's National Bureau of Agricultural Commodity and Food Standards
AD	<i>Acheta domesticus</i>

AdDV	<i>Acheta domesticus</i> Densovirus
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
ADME	Absorption, distribution, metabolism and excretion
AFNOR	Association Française de Normalisation
AMCase	Acidic mammalian chitinase head-space gas chromatography with nitrogen-phosphorus detection
AOAC	Association of Official Analytical Chemists
APGC–MS/MS	Atmospheric Pressure Gas Chromatography Tandem Mass Spectrometry
BIOHAZ	Panel on Biological Hazards
bw	body weight
CEN	Comité Européen de Normalisation
CFU	Colony Forming Units
CHL-IU	Chinese Hamster Lung
COI: 16S	Cytochrome C Oxidase Subunit 1
CONTAM	Panel on Contaminants in the Food Chain
Cox1	Cytochrome C Oxidase Subunit 1
CrPV	Cricket Paralysis Virus
DIAAS	Digestible Indispensable Amino Acid Score
DietEx	Dietary Exposure tool
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
FSANZ	Food Standards Australia New Zealand
GBIF	Global Biodiversity Information Facility
GC–MS/MS	Gas Chromatography/tandem Mass Spectrometry
GMP	Good manufacturing practice
HACCP	Hazard Analysis Critical Control Points
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
IL-13	Interleukin-13
IL-4	Interleukin-4
IM	Internal Method
ISO	International Organization for Standardization
LC-DAD	Liquid Chromatography with Diode Array Detection
LC-FDL	Liquid Chromatography - Fluorescence Detector
LC–MS/MS	Liquid Chromatography–tandem Mass Spectrometry
LOQ	Limit of Quantification
Mn	Manganese
MRLs	Maximum residue levels
MUFA	Monounsaturated Fatty Acids
N	Nitrogen
ND	Not Detected
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NEN	Netherlands Standardization Institute
NF	Novel Food
NIF	Nutrition & Food Innovation Unit
NMKL	Nordic-Baltic Committee on Food Analysis
OECD	Organisation for Economic Co-operation and Development
P95	95th percentile
PCBs	Polychlorinated Biphenyls
PDR	People's Democratic Republic
PFAS	Per- and polyfluoroalkyl substances
PmergDNV	<i>Penaeus merguensis</i> densovirus
PUFA	Polyunsaturated Fatty Acids
rRNA	Ribosomal RNA
rrnL	Large subunit ribosomal RNA
SAA	Sulfur-containing amino acids
SC	Scientific Committee
TAMC	Total Aerobic Microbial Count
TYMC	Total Yeast and Mould Count
US FDA	United States Food and Drug Administration
US FDA/CFSAN	U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition
UV	Ultraviolet
w/w	weight per weight

WG Working Group

WHO-PCDD/F-PBC-TEQ Sum of Polychlorinated Dibenzodioxins, Polychlorinated Dibenzofurans, Polychlorinated Biphenyls expressed as World Health Organization toxic equivalent.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2018-00543

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Indispensable Amino acid content and recommended amino acid scoring patterns

Amino acid (mg/g protein)	AD frozen		AD dried		AD powder		Age group		
	Min	Max	Min	Max	Min	Max	Infant (birth to 6 months) ^a	Child (6 months to 3 year) ^b	Other child, adolescent, adult ^c
Histidine	28.1	32.8	28.0	31.5	25.6	26.7	21	20	16
Isoleucine	49.6	56.9	49.4	56.0	45.0	47.6	55	32	30
Leucine	91.8	105.5	90.0	100.8	80.8	85.9	96	66	61
Lysine	69.7	80.4	58.4	79.4	53.1	64.5	69	57	48
Threonine	48.9	54.3	47.4	49.6	42.9	45.0	44	31	25
Tryptophan	13.4	14.7	12.4	12.8	11.6	12.2	17	8.5	43
Valine	71.0	81.7	73.4	79.4	66.5	80.8	55	43	40
Methionine + Cysteine (SAA)	29.5	35.5	30.0	32.9	28.5	29.9	33	27	23
Phenylalanine + Tyrosine (AAA)	103.1	116.5	105.9	120.4	100.5	107.0	94	52	41

Abbreviations: AAA, aromatic amino acids; SAA, sulfur-containing amino acids.

^aRecommended amino acid scoring patterns for infant (birth to 6 months).

^bRecommended amino acid scoring patterns for children (6 months to 3 years).

^cRecommended amino acid scoring patterns for other child, adolescents and adults.

APPENDIX B**Fatty acid profile analysis of the NF**

Fatty acids (% of total fatty acids)	AD frozen						AD dried						AD powder				
	#2	#3	#4	#5	#6	#7	#2	#3	#4	#5	#6	#7	#1	#2	#3	#4	#5
C18:0 Stearic acid	11.17	11.17	11.10	11.08	9.01	17.67	11.18	11.10	11.35	11.04	10.21	11.05	10.72	10.51	10.53	10.52	10.61
C18:1 Oleic acid	22.16	22.23	22.64	22.31	18.81	15.01	23.53	22.69	22.64	22.78	24.06	24.3	23.58	23.19	23.25	23.87	23.46
C18:2 Linoleic acid	33.57	32.93	32.93	33.07	41.39	12.19	29.49	29.19	30.23	29.11	31.42	28.24	31.3	30.67	30.75	29.7	30.72
C18:3n3 Alpha linolenic acid	2.09	2.04	2.08	2.07	1.99	<0.05	1.82	1.74	1.83	1.78	2.07	2.16	2.07	2.02	2.00	1.94	1.99
Saturated fatty acids	39.91	40.1	40.0	40.36	33.82	66.94	43.62	43.57	43.40	43.43	40.32	42.53	40.76	41.26	41.26	41.46	40.66
Monounsaturated fatty acids	23.44	23.53	24.01	23.65	20.00	18.07	24.73	24.03	23.83	24.11	25.30	25.7	24.95	24.66	24.66	25.57	24.82
Polyunsaturated fatty acids	36.02	35.33	35.35	35.47	45.25	13.99	31.31	31.71	32.37	31.66	33.72	31.04	33.93	33.29	33.33	32.20	33.75
Trans fatty acids	0.63	0.61	0.64	0.62	0.72	<0.05	0.34	0.58	0.39	0.61	0.57	0.65	0.36	0.54	0.56	0.57	0.55
Omega-3 fatty acids	2.26	2.25	2.24	2.22	2.40	<0.05	1.82	1.74	1.83	1.78	2.22	2.32	2.31	2.13	2.11	2.05	2.16
Omega-6 fatty acids	33.57	32.93	32.93	33.07	41.54	13.99	29.49	29.49	30.23	29.42	31.42	28.36	31.3	30.67	30.75	29.70	30.96
C16:0 Palmitic acid	27.41	27.66	27.50	27.66	23.00	46.94	30.89	30.82	30.66	30.33	28.76	29.99	28.58	28.71	28.70	28.49	28.6

APPENDIX C

Concentration of minerals in the NF forms, analysed by ICP-MS

		AD frozen									
Minerals (mg/kg)	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Sodium (Na)		1320	1250	1240	1360	/	/	/	860 ^{#41}	1400	
Magnesium (Mg)	350	360	340	350	350	/	/	/	/	/	
Phosphorus (P)	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/	
Potassium (K)	3500	3400	3500	3400	3500	/	/	/	/	/	
Calcium (Ca)	920	810	800	850	850	/	/	/	/	/	
Selenium (Se)	/	/	/	/	/	0.39	0.41	0.40	0.39	0.40	
Manganese (Mn)	/	/	/	/	/	29	32	53	29	29	
Iron (Fe)	50	42	40	41	42	/	/	/	/	/	
Copper (Cu)	/	/	/	/	/	17	18	16	16	17	
Zinc (Zn)	/	/	/	/	/	80	81	80	82	82	
Molybdenum (Mo)	/	/	/	/	/	0.31	0.36	0.33	0.35	0.33	
Boron (B)	/	/	/	/	/	<0.5	<0.5	<0.5	<0.5	0.67	
		AD dried									
Minerals (mg/kg)	#12	#13	#14	#15	#16	#17	#18	#19	#20	#49	
Sodium (Na)	3930	3970	4210	3880	/	/	/	/	3700	3550	
Magnesium (Mg)	1100	1100	1100	1000	/	/	/	/	/	860	
Phosphorus (P)	7.80	7.80	< 10	7.5	/	/	/	/	/	8100	
Potassium (K)	10,000	11,000	10,000	10,000	/	/	/	/	/	9700	
Calcium (Ca)	3100	3200	3000	2800	/	/	/	/	/	3100	
Selenium (Se)	/	/	/	/	1.0	1.0	1.0	1.1	1.0	/	
Manganese (Mn)	/	/	/	/	75.0	65.0	66.0	66.0	64.0	/	
Iron (Fe)	140	150	150	140	/	/	/	/	/	86	
Copper (Cu)	/	/	/	/	/	/	/	/	/	/	
Zinc (Zn)	270 ^{#50}	240 ^{#51}	210 ^{#52}	250 ^{#53}	/	/	/	/	/	250	
Molybdenum (Mo)	/	/	/	/	/	/	/	/	/	/	
Boron (B)	/	/	/	/	/	/	/	/	/	/	
		AD powder									
Minerals (mg/kg)	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30	
Sodium (Na)	4220	3930	4050	4010	3930	/	/	/	/	/	
Magnesium (Mg)	930	920	900	920	910	/	/	/	/	/	
Phosphorus (P)	< 10	< 10	< 10	< 10	< 10	/	/	/	/	/	
Potassium (K)	11,000	11,000	11,000	11,000	11,000	/	/	/	/	/	
Calcium (Ca)	2400	2300	2300	2200	2300	/	/	/	/	/	
Selenium (Se)	/	/	/	/	/	1.10	0.98	0.97	1.00	1.00	
Manganese (Mn)	/	/	/	/	/	90	67	67	99	63	
Iron (Fe)	120	110	110	110	110	/	/	/	/	/	
Copper (Cu)						/	/	/	/	/	
Zinc (Zn)	270 ^{#59}	290 ^{#60}	260 ^{#61}	260 ^{#62}	270 ^{#63}	/	/	/	/	/	
Molybdenum (Mo)	/	/	/	/	/	/	/	/	/	/	
Boron (B)	/	/	/	/	/	/	/	/	/	/	

Notes: Boron is considered a non-nutrient. #50; #51; #52; #53 analyses were conducted on different batches.

Abbreviation: ICP-MS, inductively coupled plasma mass spectrometry.

APPENDIX D

Concentration of vitamins in the NF

Vitamin	Unit	Batch number					Analytical method
		#1	#2	#3	#4	#5	
AD Frozen							
Vitamin A (retinol)	µg/100 g	<21	<21	<21	<21	<21	LC-DAD (EN 12823–12014)
Vitamin B1 mg/100 g	mg/100 g	0.91	0.82	0.95	0.80	0.86	LC-FDL (EN 14122:2014 modified)
Vitamin B2	mg/100 g	4.72	4.87	5.10	5.44	5.21	LC-FDL (EN 14152:2014 modified)
Vitamin B3 (niacin)	mg/kg	38.7	41.1	42.6	39.6	39.7	LC-FDL (EN 15652:2009)
Vitamin B12 (cyanocobalamin)	µg/100 g	1.69	1.94	1.97	1.85	1.83	LC-UV/DAD (J, AOAC 2008, vol 91 No 4)
Vitamin C (ascorbic acid)	mg/100 g	<0.5	<0.5	<0.5	<0.5	<0.5	LC-DAD (ISO 20635:2018)
Vitamin E (alpha-tocopherol)	mg/100 g	1.87	2.00	2.00	2.12	1.83	LC-FDL (EN 12822:2014)
		#6	#7	#8	#9	#10	
Vitamin B6	mg/100 g	0.14	0.16	0.13	0.15	0.17	LC-FDL (EN 14164:2014)
Vitamin B9 (folate)	µg/100 g	109	168	145	105	105	Nephelometry (NMKL 111:1985)
Vitamin D3 (cholecalciferol)	µg/100 g	1.22	0.69	0.91	0.83	0.91	LC-DAD (EN 12821:2009)
AD dried							
		#11	#12	#13	#14	#15	
Vitamin A (retinol)	µg/100 g	<21 ^{#16}	<21	<21	<21	<21	LC-DAD (EN 12823–12,014)
Vitamin B1 (thiamin)	mg/100 g	0.36	0.39	0.37	0.34	0.38	LC-FDL (EN 14122:2014 modified)
Vitamin B2 (riboflavin)	mg/100 g	12.4	13.2	14.4	12.2	13.4	LC-FDL (EN 14152:2014 modified)
Vitamin B3 (niacin)	mg/kg	109	110	114	111	113	LC-FDL (EN 15652:2009)
Vitamin B12 (cyanocobalamin)	µg/100 g	5.03 ^{#16}	4.67	4.78	4.69	4.40	LC-UV/DAD (J, AOAC 2008, vol 91 No 4)
Vitamin C (ascorbic acid)	mg/100 g	<0.5 ^{#16}	<0.5	<0.5	<0.5	<0.5	LC-DAD (ISO 20635:2018)
Vitamin E (alpha-tocopherol)	mg/100 g	4.54	4.78	5.16	4.48	4.91	LC-FDL (EN 12822:2014)
		#16	#17	#18	#19	#20	
Vitamin B6 (pyridoxine)	mg/100 g	0.16	0.18	0.16	0.16	0.15	LC-FDL (EN 14164:2014)
Vitamin B9 (folate)	µg/100 g	442	460	410	361	385	Nephelometry (NMKL 111:1985)
Vitamin D3 (cholecalciferol)	µg/100 g	0.84	1.54	1.43	1.18	1.41	LC-DAD (EN 12821:2009)
AD powder							
		#21	#22	#23	#24	#25	
Vitamin A (retinol)	µg/100 g	<21	<21	<21	<21	<21	LC-DAD (EN 12823–12,014)
Vit B1 (thiamin)	mg/100 g	1.45	1.49	1.41	1.42	1.38	LC-FDL (EN 14122:2014 modified)
Vit B2 (riboflavin)	mg/100 g	7.99	7.64	7.34	7.55	7.70	LC-FDL (EN 14152:2014 modified)
Vitamin B12 (cyanocobalamin)	µg/100 g	2.7	2.61	2.81	2.79	2.91	LC-UV/DAD (J, AOAC 2008, vol 91 No 4)
Vitamin C (ascorbic acid)	mg/100 g			<0.5	<0.5	<0.5	LC-DAD (ISO 20635:2018)
Vitamin E (alpha-tocopherol)	mg/100 g	10.4 ^{#26}	3.89	4.01	4.26	4.43	LC-FD (EN 12822:2014)
		#26	#27	#28	#29	#30	
Vitamin B3 (niacin)	mg/kg	108 ^{#24}	110 ^{#25}	84.9	86.4	85.1	LC-FDL (EN 15652:2009)
Vitamin B6 (pyridoxine)	mg/100 g	0.20	0.18	0.19	0.17	0.18	LC-FDL (EN 14164:2014)
Vitamin B9 (folate)	µg/100 g	387	427	387	382	341	Nephelometry (NMKL 111:1985)
Vitamin D3 (cholecalciferol)	µg/100 g	1.12	1.25	1.15	0.98	1.48	LC-DAD (EN 12821:2009)

Abbreviations: AOAC, Association of Official Analytical Chemistry; EN, European Standard; ISO, International Organization for Standardization; LC-DAD, Liquid Chromatography - Diode Array Detector; LC-FDL, Liquid Chromatography - Fluorescence Detector; LC-UV/DAD, Liquid Chromatography - Ultraviolet/Diode Array Detector; NMKL, Nordic-Baltic Committee on Food Analysis.

ANNEX A**Estimates of dietary exposure 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://doi.org/10.2903/j.efsa.2024.9101>).