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Safety of pea and rice protein fermented by Shiitake (*Lentinula edodes*) mycelia as a Novel food pursuant to Regulation (EU) 2015/2283

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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 pea and rice protein fermented by Shiitake mushroom (*Lentinula edodes*) mycelia as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is a mixture of fermented pea and rice protein concentrates (65% and 35%, respectively). The NF is proposed to be used as a food ingredient in specific food categories. The target population is the general population. The major constituent of this NF is protein (≥ 75% dry weight), which is well digestible and provides sufficient amounts of essential amino acids. Although a tolerable upper intake level (UL) has not been derived for protein, the protein intake from the NF may nevertheless further contribute to an already high dietary protein intake in Europe. The Panel notes that the cumulative exposure to the nutrients and contaminants analysed does not raise concern. The reported values for the levels of antinutritional factors in the NF are comparable to those in other foodstuffs. 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. No toxicological studies with the NF were provided by the applicant; however, the Panel considers that no toxicological studies are required for this NF. The NF has the potential capacity to sensitise individuals or to induce allergic reactions in individuals allergic to pea, rice and Shiitake mushroom. However, this risk is expected not to be higher than that resulting from the normal consumption of pea, rice or the fruiting body of the Shiitake mushroom. The Panel considers that the NF is safe at the proposed conditions of use.

Keywords: Novel Foods, food safety, pea protein, rice protein, *Lentinula edodes*, Shiitake

Requestor: European Commission

Question number: EFSA-Q-2020-00040

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1. Introduction

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

On 12 December 2019, the company MycoTechnology, Inc., submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283 to place on the EU market pea and rice protein fermented by Shiitake mushroom (*Lentinula edodes*) mycelia as a novel food.

Pea and rice protein fermented by Shiitake mushroom (*Lentinula edodes*) mycelia is intended to be used in a number of foods.

The applicant has requested data protection according to the provisions of 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 pea and rice protein fermented by Shiitake mushroom (*Lentinula edodes*) mycelia as a novel food.

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 the Commission Implementing Regulation (EU) 2017/2469[1].

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 of the available (proprietary, confidential and published) scientific data (both 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 NF production process and information concerning the composition, recipe and analysis of the novel food.

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 the Commission Implementing Regulation (EU) 2017/2469.

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 pea and rice protein fermented by Shiitake (*Lentinula edodes*) mycelia.

The NF falls under category (ii), i.e. food consisting of, isolated from or produced from microorganisms, fungi or algae according to Article 3(2)(a) of Regulation (EU) No 2015/2283.

The NF is produced by the fermentation with Shiitake mycelia of pea and rice protein and consists of $\geq$ 75% protein on a dry basis and an estimated level of Shiitake mycelia biomass of $<$ 0.1 weight (wt) %.

[1] 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.

The NF is proposed to be used as food ingredient in specific food categories. The target population proposed by the applicant is the general population.

3.2. Identity of the NF

The NF is the heat-treated mixture of pea and rice protein concentrates (65% pea protein and 35% rice protein) obtained after fermentation by Shiitake mycelia (*Lentinula edodes*), in a powder form. The fermentation is performed to improve organoleptic properties of the pea and rice protein starting materials.

The fungus *Lentinula edodes* (basidiomycete family) is listed in the Index fungorum,[2] and it is commonly known by its Japanese name 'Shiitake'. Under the conditions of use in aqueous culture, the mushrooms *Lentinula edodes* grow as vegetative form. According to the applicant, the organism used in the production process of the NF is *Lentinula edodes* vegetative form (also identified as Shiitake mycelia).

The strain of *Lentinula edodes* used to produce the NF was originally obtained from Pennsylvania State University, ID No. WC 1008 (https://plantpath.psu.edu/facilities/mushroom/cultures/spawn).

The taxonomic identification of the strain as *Lentinula edodes* was established by comparing the highly conserved Internal Transcribed Spacer (ITS) region of *L. edodes* to the genetic sequences in the NCBI public database (GenBank: AB366150.1) and confirmed as 100% identity to *L. edodes*.

3.3. Production process

According to the information provided, the NF is produced following good manufacturing practice (GMP) and hazard analysis critical control points (HACCP) principles.

Flow charts of the manufacturing process with detailed description of the methods involved at each step, a complete list of the culture media constituents and processing aids and the respective certificates of analysis were provided by the applicant.

The starting materials include pea protein concentrates and rice protein concentrates. Further information on the origin, specifications, certificates of analysis and process flow-charts from the protein concentrates suppliers were provided by the applicant. Additional starting materials include also processing aids (e.g. maltodextrin, carrot powder, anti-foam agent) and the mycelium from Shiitake.

The production process refers to a closed system that includes subsequent fermentation steps.

In short, an inoculum *of L. edodes* (Shiitake mycelia) is made by successive fermentation steps in liquid media at a certain temperature and pH in order to prepare for the inoculation of the main fermenter. The main fermentation is conducted at specific conditions, where the *L. edodes* biomass is mixed with 65% pea protein and 35% rice protein concentrates, while slowly stirring for up to 40 h. The growth of the shiitake biomass between fermentations is quantified by pH monitoring and visual appearance.

The production process for the fermented pea and rice protein concentrates by *L. edodes* consists of fermentation followed by heat treatment steps and spray-drying. The powder is packaged into poly-lined kraft bags, heat-sealed and stored at ambient conditions.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.4. Compositional data

The NF is a heat-treated mixture of pea and rice protein concentrates fermented by *L. edodes*, and the major constituents are proteins ≥ 75% (dry weight), produced from a mixture which consists of a combination of 65% pea protein concentrate and 35% rice protein concentrate and remaining processing aids. The NF also contains carbohydrates, dietary fibre, fat, ash and water.

In order to confirm that the production process is reproducible and adequate to produce the NF on a commercial scale, the applicant provided analytical information for five batches of the NF (Table 1).

[2] http://www.indexfungorum.org/Names/Names.asp

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

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Crude protein (% DW)* (N-protein conversion factor 6.25)	78.11	78.48	78.47	79.71	78.45	Combustion-Dumas; AOAC 990.03; AOAC 992.15
Moisture (%)	2.70	3.00	1.80	2.70	2.80	Gravimetric method AOAC 925.09; 985.14 Vacuum Oven
Ash (% DW)	3.25	3.08	3.44	6.21	6.33	Gravimetric method AOAC 942.05
Total fat (% DW)	8.19	5.33	9.68	6.62	6.84	GC-FID AOAC 996.06 internal adaption
Carbohydrates (%DW)	10.45	13.13	8.40	7.46	8.38	By calculation
Total dietary fibre (%)	12.1	13	12.5	11.6	12.1	AOAC 991.43 Mod.
Total sugars (%)	1.06	1.02	0.65	0.94	0.62	AOAC 982.14,
Water activity (a_w)	0.120	0.462	0.103	0.127	N.A.	AOAC 978.18 mod.
Potassium (%)	0.06	0.07	0.06	0.06	0.06	ICP, AOAC 984.27 mod.927.02
Sodium (%)	0.66	0.63	0.68	0.69	0.58	
Calcium (%)	0.03	0.04	0.03	0.03	0.03	
Iron (%)	0.02	0.01	0.02	0.01	0.02	
Vitamin D2 (µg/100 g)*	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC-MS/MS
Vitamin D3 * (µg/100 g)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	

DW: Dry weight; AOAC: Association of Official Analytical Chemists; GC-FID: Gas Chromatography with Flame Ionisation Detection; LC-MS/MS: Liquid chromatography with tandem mass spectrometry; ICP: Inductively coupled plasma.
*: < 4 IU/100 g 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).

The applicant also provided a detailed description of the amino acid composition (See Section '3.9 Nutritional information').

The appearance of the batches was that of a light-tan powder, as confirmed by colorimetric analysis.

The level of contaminants was also monitored in the NF. Analytical information on chemical and microbiological parameters for five batches of the NF was also provided (Table 2).

Pesticides residues were below the limit of detection and glyphosate was reported at levels between 0.01 and 0.05 mg/kg which is under the lowest MRL (0.05 mg/kg).

Table 2: Batch-to-batch analysis of chemical contaminants and microbiological parameters in the NF

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Mycotoxins						
Aflatoxin B1 (µg/kg)	< 0.5	< 0.5	< 0.5	0.619	< 0.5	AOAC 999.07 Modified (UHPLC-MS/MS)
Aflatoxin B2 (µg/kg)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Aflatoxin G1 (µg/kg)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Aflatoxin G2 (µg/kg)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Aflatoxin total (B1 + B2 + G1 + G2) (µg/kg)	< 2	< 2	< 2	2.119	< 2	
Deoxynivalenol (µg/kg)	< 100	< 100	< 100	< 100	< 100	Internal Method (UHPLC-MS/MS)

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Fumonisin B1 (μg/kg)	< 25	< 25	< 25	< 25	< 25	Anal. Bioanal. Chem (2012) 402:2675-2686 (UHPLC-MS/MS)
Fumonisin B2 (μg/kg)	< 25	< 25	< 25	< 25	< 25	
HT-2 toxin (μg/kg)	< 100	< 100	< 100	< 100	< 100	
Ochratoxin A (μg/kg)	2.17	< 1	< 1	< 1	< 1	
T-2 toxin (μg/kg)	< 10	< 10	< 10	< 10	< 10	
Zearalenone (μg/kg)	< 30	< 30	< 30	< 30	< 30	
Heavy metals						
Arsenic (mg/kg)	0.02	0.02	0.02	0.03	0.02	ICP-MS[1]
Lead (mg/kg)	0.04	0.04	0.04	0.02	0.04	
Cadmium (mg/kg)	0.03	0.02	0.03	0.03	0.02	
Mercury (mg/kg)	0.01	< 0.005	< 0.005	0.01	0.01	
Microbiological						
Aerobic Plate Count (CFU/g)	< 10	< 10	< 10	< 10	290	AOAC 966.23
Yeast (CFU/g)	< 10	< 10	< 10	< 10	< 10	FDA-BAM, Ch 18; AOAC 997.02
Mould (CFU/g)	< 10	< 10	200	< 10	< 10	FDA-BAM, Ch 18; AOAC 997.02
Coliforms (CFU/g)	< 10	< 10	< 10	< 10	< 10	AOAC 991.14
E. coli (CFU/g)	< 10	< 10	< 10	< 10	< 10	AOAC 991.14
S. aureus (CFU/g)	< 10	< 10	< 10	< 10	N.A.	AOAC 2003.07
B. cereus (CFU/g)	/	20	10	< 10	N.A.	FDA BAM chapter 14
Salmonella spp. (in 25 g)	Not detected	Not detected	Not detected	Not detected	Not detected	AOAC 2016.01
Listeria spp. (in 25 g)	Not detected	Not detected	Not detected	Not detected	Not detected	AOAC 2004.06 AOAC 2016.07

AOAC: Association of Official Analytical Chemists; FDA BAM: FDA's Bacteriological Analytical Manual; N.A: Not available; /: not provided; ICP-MS: inductively coupled plasma-mass-spectrometry; UHPLC-MS/MS: Ultra-high-performance liquid chromatography tandem mass spectrometry; 1: Method reference: J. AOAC (2007) v. 90: 844-856 (modified).

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

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

3.4.1. Stability

The applicant performed two separate stability studies with two independently produced batches of the NF. The tests were carried out under accelerated conditions at 40°C and at 75% RH for a period of 24–32 weeks (168–224 days). In the first study, the samples of the NF were taken every 2 weeks for 168 days and were analysed for pH, colour, moisture, protein and nitrogen solubility at a pH ranging from 3 to 7. In the second study, samples of a second batch of the NF were taken on days 0, 112, 166 and 224, and analysed for pH, colour, moisture, protein, proximates, amino acid profile, microbiological contaminants and nitrogen solubility at pH 7. The NF was found to be stable under accelerated storage conditions for 168–224 days. The applicant provided certificates of analysis of both studies as well as laboratory accreditations.

From the results of the stability studies, the applicant proposed a shelf-life for the NF of 2 years at 25°C.

Upon EFSA's request to prove the shelf-life of 2 years, the applicant performed tests under accelerated conditions at 40°C and at 75% RH and used a lot which was 659 days old as starting material to simulate a total real-time storage of 3.5 years. The samples were taken at baseline (5 days), 9.6 weeks, 18.1 weeks, 27.1 weeks and were analysed for proximates, colour, pH, lipid oxidation (thiobarbituric acid, hexanal), microbiological parameters (APC, mould, yeast, *E. coli*, Coliforms, *S. aureus*, *B. cereus*), sensorial analysis (Table 3).

Table 3: Stability of the NF

Parameters	**NF**				
Time points (days)	**Baseline 5 days**	**Baseline 659 Days**	**Baseline + Accelerated for 8 Weeks (877 days)**	**Baseline + Accelerated for 16 Weeks (1072 days)**	**Baseline + Accelerated for 24 Weeks (1277 days)**
Microbiological (cfu/g)					
Aerobic Plate Count (APC)	< 10	< 10	< 10	< 10	< 10
Yeasts	< 10	< 10	< 10	< 10	< 10
Moulds	< 10	< 10	< 10	< 10	< 10
S. aureus	N.A.	N.A.	< 10	< 10	< 10
Coliforms	< 10	< 10	< 10	< 10	< 10
E. coli	< 10	< 10	< 10	< 10	< 10
L. monocytogenes (in 25 g)	ND	ND	ND	ND	ND
B. cereus	N.A.	N.A.	< 10	< 10	< 10
Salmonella (in 25 g)	ND	ND	ND	ND	ND
Physical/Chemical parameters					
Moisture (% m/m)	2.59	2.80	4.20	4.69	5.50
pH	6.10	6.03	5.96	5.82	5.78
Protein (measured) %	76.81	76.94	76.19	73.63	75.19
Carbohydrates (%)	N.A.	4.07	8.45	4.69	7.29
Fat (%)	N.A.	8.59	6.86	9.00	8.52
Ash (%)	N.A.	7.60	4.30	8.08	3.83
Calories (kcal/100 g)	N.A.	401	400	394	404
TBA – Rancidity (mg/kg)	N.A.	2.46	N.A.	2.26	N.A.
Hexanal (ppm)	N.A.	38.8	N.A.	19.8	N.A.

N.A: Not analysed – not provided, ND: Non-detected.

The applicant explained that for calculating the simulated age of the product, they used a factor of 3.25 based on the principle of the Arrhenius equation (Arrhenius Equation, 2021), to the days the product was stored at accelerated conditions.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for 2 years.

Upon EFSA's request of information on stability in intended food matrix, the applicant provided a stability study of the NF as an ingredient in plant-based burger meat analogue, plant-based whole milk analogue (unflavoured) and chocolate chip muffin. The burger meat and whole milk analogues with the NF were refrigerated at 5°C (% RH not recorded but expected to be standard for commercial refrigerated coolers) whereas the chocolate chip muffin with the NF was stored at (25°C/60%RH). Samples were collected at baseline (0 days), 5/7 days and 10 days and analysed for the following: visual appearance, acrylamide, proximates (carbohydrates, protein content, fatty acids profile,

moisture, ash), heavy metals, microbiological parameters and amino acid concentrations and showed no changes. Microbiological stability in the matrix was also assessed (milk analogue, plant-based burger and baked chocolate chip muffins). The results showed an increase in microbiological parameters compatible with microbial growth during storage of highly perishable food matrices. The Panel notes that the NF itself has very low total microbial counts, and low water activity and hence, the microbiological levels in the representative foods are more related to the quality level of the food itself (matrix) rather than that of the NF.

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

3.5. Specifications

The specifications of the NF are indicated in Table 4.

Table 4: Specifications of the NF

Description: powder from pea and rice protein fermented by Shiitake (*Lentinula edodes*) mycelia	
Parameter	**Specification**
Protein, % dry weight (DW) basis	≥ 75.0%
Moisture	≤ 7%
Total Fat	≤ 10%
Ash	≤ 10%
Carbohydrates (by calculation)	≤ 15%
Mycotoxins	
Aflatoxin B1	< 1 μg/kg
Aflatoxin B2	< 1 μg/kg
Aflatoxin G1	< 1 μg/kg
Aflatoxin G2	< 1 μg/kg
Aflatoxin total (B1 + B2 + G1 + G2)	< 3 μg/kg
Heavy metals	
Arsenic	< 0.1 μg/g
Lead	< 0.3 μg/g
Cadmium	< 0.1 μg/g
Mercury	< 0.1 μg/g
Microbiological	
TAMC	< 1,000 CFU/g
TYMC	< 100 CFU/g
Coliforms	< 10 CFU/g
Salmonella spp.	Not detected in 25 g
E. coli	< 10 CFU/g
Listeria monocytogenes	Not detected in 25 g

TAMC: total aerobic microbial count; TYMC: total yeast and mould count; CFU: colony forming units.

The applicant proposes a specification limit for TAMC as < 5,000 CFU/g. The Panel notes that considering the NF production process (thermal processing) and compositional analyses of five batches (below 10 CFU/g with only one batch showing a maximum value of 290 CFU/g), a lower specification limit could be met. The Panel also notes that TAMC is considered an indicator of hygiene and that this parameter could affect the safety of the NF. Therefore, the Panel proposes a criterion for this parameter of ≤ 1,000 CFU/g.

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

3.6.1. History of use of the source

The NF is composed of pea and rice protein fermented with the mycelium of Shiitake mushroom (*L. edodes*).

Pea, rice and its protein concentrates are consumed in the EU. Pea (*Pisum sativum*) is a legume in the family Fabaceae, grown from its edible seeds. Pea is a crop cultivated extensively around the world and is rich in protein (23–31%). Pea proteins have a good capacity for fat/water binding, allowing adequate texture, emulsification and gelation and are being proposed as a possible alternative protein in different food products (Bessada et al., 2019). Rice is the seed of the grass species *Oryza sativa* (plant family Poaceae (Gramineae)) which has its origin in Asia. Rice is a cereal widely consumed in the EU and around the world. Rice protein content is 6–10% depending on cultivation conditions and rice variety.

According to the applicant, the starting materials rice and pea protein concentrates used to produce the NF contain > 80% protein and have been recognised by the U.S. FDA as GRAS for use in food (GRN 609[3]; GRN 608[4]).

The mycelium of the fermentation organism (*L. edodes*) used for the production of the NF was not used for food production within the Union prior to 15 May 1997, although the fruiting body of *L. edodes* has been consumed all over Europe long before. *L. edodes* is cultivated and consumed in many Asian countries, as well as being dried and exported to many countries around the world. It is a feature of many Asian cuisines including Chinese, Japanese, Korean and Thai in both fresh and dried forms (EFSA NDA Panel, 2010).

3.6.2. History of use of the NF

According to the applicant, the NF is authorised as a food ingredient in the United States.[5]

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 NF is proposed by the applicant to be used as an ingredient in a number of food products. These food products are defined using the FoodEx2 hierarchy (EFSA, 2015), and the maximum use levels are reported in Table 5.

The Panel notes that the proposed uses of the NF include food categories that are generally not considered as protein sources.

Table 5: Food categories and maximum use levels intended by the applicant

Proposed food category & use	FoodEx2 level	FoodEx2 code	Food category (Foodex2)	Max use level (g NF/100 g)
Baked goods-bread & rolls	4	A005R	Gluten-free bread	5
	3	A005Z	Extruded, pressed or puffed bread	5
	3	A04KZ	Unleavened or flat bread and similar	5
	3	A0BY0	Leavened bread and similar	5
	4	A007A	Breadcrumbs	5
	4	A007B	Croutons[(a)]	5
	4	A007C	Bread stuffing[(a)]	5
	5	A03ZC	Sandwich with fish topping/filling[(a)]	5

[3] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=609
[4] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=608
[5] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=794

Proposed food category & use	FoodEx2 level	FoodEx2 code	Food category (Foodex2)	Max use level (g NF/100 g)
	5	A03ZD	Sandwich with vegetable topping/filling[(a)]	5
	5	A03ZE	Sandwich with cheese and vegetable topping/filling[(a)]	5
	5	A03ZH	Sandwich with fish and vegetable topping/filling[(a)]	5
	5	A03ZP	Pizza and similar with cheese topping[(a)]	5
	5	A03ZR	Pizza and similar with fish/seafood topping[(a)]	5
	5	A03ZS	Pizza and similar with vegetable topping[(a)]	5
	5	A03ZT	Pizza and similar with cheese, and vegetables[(a)]	5
	5	A03ZV	Pizza and similar with cheese, and vegetables and fruits[(a)]	5
	5	A03ZZ	Pizza and similar with cheese, and mushrooms[(a)]	5
	5	A03ZA	Sandwich with cheese topping/filling[(a)]	5
	5	A03ZB	Sandwich with processed meat topping/filling[(a)]	5
	5	A03ZG	Sandwich with meat and vegetable topping/filling[(a)]	5
	5	A03ZJ	Doner kebab[(a)]	5
	5	A03ZQ	Pizza and similar with processed meat topping[(a)]	5
	5	A03ZX	Pizza and similar with meat, and vegetables[(a)]	5
	5	A03ZY	Pizza and similar with cheese, meat and vegetables[(a)]	5
	5	A040A	Pizza and similar with cheese, meat and mushrooms[(a)]	5
	5	A040B	Pizza and similar with cheese, meat, mushrooms, and vegetables[(a)]	5
Baked goods-bars and grain-based bars	4	A00EZ	Cereal bars plain	33
	4	A00FA	Cereal bars mixed	33
Beverages-Juices & nectars	3	A03BB	Fruit nectars (min. 25–50% fruit as defined in EU legislation)	1.04
	4	A03BN	Fruit juice concentrates[(b)]	5.2
	4	A03CG	Dehydrated/powdered fruit juice[(b)]	5.2
	4	A03CZ	Vegetable juice concentrate[(c)]	100
	4	A03DB	Mixed fruit and vegetable juices[(d)]	10.5
	5	A03DH	Multivitamin juices[(d)]	10.5
	3	A04PQ	Vegetable juices	20
	5	A04PS	Other (mixed) fruit and vegetable juices or nectars	10.5
	3	A0BY4	Fruit juices (100% from named source)	1.04
Beverages – smoothies	5	A03DF	Fruit smoothies	20
Beverages-Ready-to-mix beverage Powder	4	A03GF	Powdered drink bases[(e)]	93.3
Breakfast cereals	3	A00EJ	Muesli and similar mixed breakfast cereals	33
	3	A04LK	Processed and mixed breakfast cereals	33
Confectionary	5	A034R	Chocolate-coated confectionery	7
Dairy product analogues	4	A02QB	Ice cream, milk-imitate based	11
	5	A03TR	Imitation cream	11
	5	A03TS	Non-dairy coffee creamer	11
	5	A03TY	Imitation cheese	11
	4	A01BK	Coconut milk (*Cocos nucifera*) liquid	11
	4	A03TH	Milk imitates	11
	4	A03RV	Single meal replacement for weight reduction (non-milk based)	11

Proposed food category & use	FoodEx2 level	FoodEx2 code	Food category (Foodex2)	Max use level (g NF/100 g)
Salad dressings	4	A03YV	Mushroom salad[(a)]	26
	4	A042C	Mixed green salad[(a)]	26
	4	A042D	Mixed vegetable salad[(a)]	26
	4	A042E	Caesar salad[(a)]	26
	4	A042F	Greek salad[(a)]	26
	4	A042G	Prepared legume (beans) salad*	26
	4	A042J	Prepared rice salad[(a)]	26
	4	A042K	Prepared nut salad[(a)]	26
	4	A042L	Prepared meat salad[(a)]	26
	4	A042M	Prepared mixed egg/meat/fish/vegetable salad[(a)]	26
Processed meat and meat products	2	A024B	Canned-tinned meat	14
	2	A024F	Sausages	14
	2	A026J	Meat specialties	14
	5	A03VZ	Meat in aspic	14
	5	A03XA	Meat loaf	14
	5	A03XF	Meat burger (no sandwich)	14
	5	A03XG	Meat balls	14
	5	A03XH	Meat terrine	14
	5	A03YP	Omelette with bacon[(a)]	14
	5	A040D	Sausage roll[(a)]	14
	5	A040J	Meat-based canapé[(a)]	14
	2	A04ND	Processed whole meat products	14
	5	A03ZK	Hot dog with bread[(a)]	14
	5	A03ZL	Hamburger with bread[(a)]	14
Flavoured milk drinks	4	A02MP	Flavoured milks	1.04
Meal replacements	4	A03RV	Single meal replacement for weight reduction	1.04
Yoghurt	4	A02NE	Yoghurt	5
	4	A02NQ	Yoghurt drinks, including sweetened and/or flavoured variants	5
	5	A03TV	Soya yoghurt	5
	5	A03TZ	Imitation yoghurt, non-soya	5
Pasta	4	A007E	Pasta, plain (not stuffed), uncooked	15
	4	A007S	Pasta-based dishes, uncooked[(a)]	15
	5	A008B	Pasta, gluten free	15
	5	A008D	Gnocchi	15
	4	A042H	Prepared pasta salad[(a)]	15
	5	A0CDP	Pasta, filled, cooked[(a)]	15
	5	A0CDQ	Pasta, plain (not stuffed), cooked	15
	4	A0ERE	Filled (stuffed) pastas[(a)]	15
Meat alternatives	3	A03TE	Meat imitates	40
	5	A03TT	Tofu	40
Prepared soups, dry soup mixes and condensed soups	3	A041L	Soups (ready-to-eat)	3.3
	3	A0B9J	Soups (dry mixture, uncooked)	3.3

(a): Recipe – only the proportion of food reflecting the amount of the target food is to be considered in the intake assessment.
(b): Applicant use level for fruit juice (1,040 mg/100 g) multiplied by 5 to account for it being a concentrate, which brings it to the volume as consumed.

(c): Applicant use level for vegetable juice (20,000 mg/100 g) multiplied by 5 to account for it being a concentrate, which brings it to the volume as consumed.
(d): Assumed that half of the beverage was fruit juice and half was vegetable juice. Fruit juice: 1040 mg/100 g use level 50%, vegetable juice: 20,000 mg/100 g use level 50%
(e): 93,300 mg of the NF per 100 g powder – (assumes 32.67 g NF per 35 g powder per serving).

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 5), 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 g/kg body weight (bw) basis and g/day), among the EU dietary surveys, are presented in Tables 6 and 7.

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 6: Intake estimates for the NF resulting from its use as an ingredient in the intended food categories at the maximum proposed use levels (g/kg bw per day)

Population group	Age (years)	Mean intake (g/kg bw per day)		P95th intake (g/kg bw per day)	
		Lowest[(b)]	Highest[(b)]	Lowest[(c)]	Highest[(c)]
Infants	< 1	0.1	1.0	0.5	3.7
Young children[(a)]	1–< 3	0.6	1.9	1.7	3.1
Other children	3–< 10	0.7	1.2	1.4	2.4
Adolescents	10–< 18	0.3	0.8	0.5	1.7
Adults[(d)]	≥ 18	0.3	0.6	0.8	1.2

(a): Referred as toddlers in the EFSA food consumption comprehensive database (EFSA, 2011).
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 22/12/2020. The lowest and the highest averages observed among all EU surveys are reported in these columns. The data relate to a period in which UK was still a Union Member State.
(c): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 22/12/2020. The lowest and the highest P95th intakes observed among all EU surveys are reported in these columns (P95th intakes based on less than 60 individuals are not considered). The data relate to a period in which UK was still a Union Member State.
(d): Includes elderly, very elderly, pregnant and lactating women.

Table 7: Intake estimates for the NF resulting from its use as an ingredient in the intended food categories at the maximum proposed use levels (g/day)

Population group	Age (years)	Mean intake (g/day)		P95th intake (g/day)	
		Lowest[(b)]	Highest[(b)]	Lowest[(c)]	Highest[(c)]
Infants	< 1	0.9	7.8	4.3	29.4
Young children[(a)]	1–< 3	6.5	26.0	17.4	35.6
Other children	3–< 10	13.4	34.2	26.6	67.0
Adolescents	10–< 18	13.8	35.0	26.9	75.2
Adults[(d)]	≥ 18	20.3	41.9	49.0	86.7

(a): Referred as toddlers in the EFSA food consumption comprehensive database (EFSA, 2011).
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 22/12/2020. The lowest and the highest averages observed among all EU surveys are reported in these columns. The data relate to a period in which UK was still a Union Member State.
(c): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 22/12/2020. The lowest and the highest P95th intakes observed among all EU surveys are reported in these columns (P95th intakes based on less than 60 individuals are not considered). The data relate to a period in which UK was still a Union Member State.
(d): Includes elderly, very elderly, pregnant and lactating women.

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

No ADME data have been provided for the NF, which is mainly composed of protein (min. 75%). The Panel considers that no ADME testing is necessary for the safety assessment of the NF.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF which consists mainly of proteins (≥ 75% dry weight) (see Section 3.4 Compositional data).

The NF is intended to be used as an ingredient in conventional food products (Section 3.7.2 Proposed uses and use levels). To address the protein quality of the NF, the applicant provided data on the amino acid profile for five non-consecutive batches of the NF (Appendix A Batch-to-batch amino acid analysis), the faecal digestibility of crude protein for deriving Protein Digestibility Corrected Amino Acid Scores (PDCAAS) as well as on the ileal digestibility of the amino acids for the calculation of the Digestibility Indispensable Amino Acid Scores (DIAAS) both for an 'unfermented blend' of pea and rice protein and the fermented pea and rice blend (the NF).

The impact of the fermentation was investigated by the applicant. The nutritional analysis indicated that the crude protein (CP) content was similar in both samples (unfermented and fermented protein blends), as well as the total content of indispensable amino acids with the exception of the concentration of lysine that was approximately 25% higher in the unfermented protein blend sample (see Appendix B Amino acid composition of the protein concentrate blends).

Ileal digestibility was studied in nine young castrated male pigs receiving unfermented and fermented (by Shiitake mycelia) blend of 65% pea protein and 35% rice protein as the sole protein source, or no protein for the determination of basal endogenous amino acid losses, in a diet in meal form providing 3.3 times the estimated energy requirement for maintenance and vitamins and minerals to meet or exceed requirement estimates for growing pigs (Unpublished report, Bailey and Stein). The animals had a T-canula in the distal ileum and were allotted to a triplicated 3 × 3 Latin square design with three pigs and three periods in each square. Diets were randomly assigned to pigs in a manner that within each square, one pig received each diet, and no pig received the same diet twice during the treatment (resulting in nine replicate pigs per treatment). All diets contained titanium dioxide as an indigestible marker. Feeding and collection of faecal and ileal digesta samples were done according to Mathai et al. (2017). Apparent ileal digestibility (AID) and standardised ileal digestibility (SID) of CP and AA were determined as described by Stein et al. (2007), and standardised total tract digestibility (STTD) of CP according to Mathai et al. (2017). Values for STTD and SID were used by the applicant to calculate PDCAAS and DIAAS, respectively.

These data submitted by the applicant (Unpublished report, Bailey and Stein) have also been included in a subsequent publication (Clark et al., 2022) as retrieved by EFSA.

Differences in the SID of CP as well as the SID of all indispensable and dispensable AA between the 'unfermented' (UF) and the 'fermented' (F) pea–rice protein concentrate blends were not identified. (Appendix C).

The PDCAAS and DIAAS values were calculated using the FAO recommended amino acid scoring patterns FAO, 2013) for the age groups 'child 6 months to 3 years' and for 'older child, adolescent, adult' (> 3 years).

PDCAAS values were found not to be different between unfermented and fermented protein blends for both age groups. For children aged 6 months to 3 years, PDCAAS values for the unfermented and fermented proteins were 86 and 91, and for older children, adolescents and adults, 101 and 108. The first limiting amino acids (AA) when compared with the amino acid scoring pattern were sulfur amino acids (SAA) and Lys for unfermented protein and fermented protein, respectively, for both age groups.

DIAAS values for 'young children' and for 'older children, adolescents, and adults' calculated for both age groups were greater ($p < 0.05$) for the fermented than for the unfermented pea–rice protein. For children aged 6 months to 3 years, the DIAAS was 70 and 86 for unfermented and fermented proteins, respectively. For older children, adolescents and adults, the DIAAS was 82 and 102 for unfermented and fermented proteins, respectively. The first limiting AAs in the proteins when compared with the AA requirements for both age groups were SAA and Lys for unfermented and fermented proteins, respectively (Clark et al., 2022; Unpublished report Bailey and Stein).

The Panel notes that based on DIAAS cut-off values describing protein quality as suggested by FAO (FAO, 2013), the NF is a protein source that can be considered a 'good' quality protein (DIAAS 75-99).

As stated in Section 3.5 on Specifications, the NF contains 75% protein. Therefore, based on the highest (P95) estimated daily intakes of the NF, the highest P95 estimated daily intakes of protein calculated both in absolute values per day (g/day) and on a per body weight basis (g/kg bw per day) are reported in Table 8.

Table 8: Estimated highest P95 of daily intake of protein from the NF as ingredient in foods and beverages considering protein concentration in the NF of 75% (calculated by EFSA)

Population group	Age (years)	Estimated High level (≥ P95) protein intake from NF Uses (g/day)[(a)]	Estimated High level (≥ P95) protein intake from NF Uses (g/kg bw per day)[(a)]	PRIs for protein (g/kg bw per day) EFSA NDA Panel (2012)[(b)]
Infants	< 1	22.1	2.8	1.14
Young children[(c)]	1–< 3	26.7	2.3	0.97
Other children	3–< 10	50.2	1.8	0.85
Adolescents	10–< 18	56.4	1.3	0.83
Adults[(d)]	≥ 18	65	0.9	0.83

NF: Novel Food.
(a): Calculated from highest values from ranges presented in Tables 6 and 7, assuming a 75% protein content in the NF (Table 4, NF Specifications). *The applicant proposes specifications for protein content ≥ 75.0%. This could result in higher protein intakes.*
(b): Lowest values from ranges presented in Table 12 of EFSA NDA Panel (2012). Intakes up to twice the PRI are considered safe for adults (EFSA NDA Panel, 2012).
(c): Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

Based on intake estimates of the NF (see Section 3.7.3), highest P95 protein intake from the NF exceeds current PRIs for protein (EFSA NDA Panel, 2012) in all age groups, especially in infants and young children where intakes may be 2.5 and 2.4 fold the PRIs (Table 8).

The Panel notes that the consumption of the NF may contribute to a further increase of an already high dietary protein intake. However, no tolerable upper intake level (UL) has been derived for protein. In addition, the Panel notes that the estimated intake of the NF were based on high consumption (95th percentile) of a large number of food categories (representing the most conservative scenario) and may overestimate the potential protein intake.

Analytical data on vitamin D and selected minerals (sodium, potassium, calcium and iron) have been provided for five batches of the NF (see Compositional data section). Regarding the reported concentrations and the proposed uses, the Panel considers that for vitamin D, and the minerals, except for iron, the contribution to the overall dietary intake is not of relevance. For iron, based on the 95th percentile intake, the iron intake from the NF (5.7, 6.9, 12.9, 14.5 and 16 mg/day in infants, young children, older children, adolescents and adults, respectively) may be close to or slightly above PRIs (EFSA NDA Panel, 2015). The Panel notes the conservative exposure estimate, that no UL has been set for iron, and the statement from SCF and EFSA NDA Panel in 2006 'that based on estimates of iron intakes in European countries, the risk of adverse effects from high iron intake from food sources, including fortified foods in some countries, is considered to be low for the population as a whole' (SCF and EFSA NDA Panel, 2006).

The NF contains < 0.1% w/w of Shiitake mycelia biomass. Macronutrients in edible Shiitake mushroom are mainly carbohydrates with lower amounts of fibre, protein and small amounts of fat. It also contains some vitamins and minerals. Considering the very small amounts of Shiitake biomass in the NF, those components are not nutritionally relevant. Moreover, the additional raw materials (i.e. maltodextrin and carrot powder) are not considered to have any adverse or antinutritional effect on consumers.

Furthermore, antinutrients in the NF were also assessed by the applicant. Upon an EFSA request, the applicant provided further information on the antinutrients that can be found in the main starting materials (pea and rice) and the presence of those antinutrients in the NF. The applicant provided references on the antinutrient phytic acid, which has been shown to chelate minerals and impair absorption of nutrients (Reddy et al., 1982; Hallberg et al., 1989; Phillippy, 2006; Schlemmer et al., 2009; Joye, 2019). The level of phytic acid was found in rice in the range of 0.06–1.08% and in peas 0.22–1.22%. The applicant provided analytical data on two batches of the NF for several antinutrients and undigestible carbohydrates and compared it with the main NF sources rice protein, pea protein and a blend pea/rice protein (Appendix D). The NF analysis for phytic acid indicated an average of 0.95%, which is within the range of the antinutrients in the normal human diet. Plant lectins are antinutrients that can be found in peas, having the potential to bind the soluble carbohydrates (Petroski and Minich, 2020). The applicant indicated that lectins cannot be quantified in the NF which is a proteinaceous matrix and therefore low levels of lectins in the NF cannot be verified. The NF

undergoes further thermal processing during the production process which is sufficient to destroy the lectin activity. Therefore, lectin (haemagglutinin binding) is not expected in the NF.

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

No toxicological studies conducted with the NF were provided. As described in Section 3.4 Compositional data, the NF is prepared from pea and rice protein fermented by *L. edodes* (Shiitake) mycelia. The primary constituents of the NF, pea and rice protein concentrates, are consumed in the EU and outside the EU. (see Section 3.6 History of use).

The NF contains < 0.1% w/w of Shiitake mycelia. Shiitake mushroom (*L. edodes)*, is an edible widely cultivated mushroom.

Literature searches performed by the applicant and by EFSA on Shiitake mushrooms mycelia retrieved no studies that were relevant for the NF toxicological assessment. Van der Molen et al. (2017) performed an assessment of mushrooms used in dietary supplements and a comparison of Shiitake mycelium and fruiting bodies used as food, reporting not substantially differences in composition (98% similarity).

Taking into account the nature of the NF and the history of safe use, the Panel considers that no toxicological studies are required on the NF.

3.10.1. Human data

No human studies conducted with the NF were provided. The applicant referred to published human studies performed with the fermentation organism used to produce the NF, *L. edodes*.

Taking into account the nature of the NF and the history of safe use, the Panel considers that no human studies are required on the NF.

3.11. Allergenicity

The NF contains protein (≥ 75%) based on protein concentrates of starting material inputs, pea and rice, and has been manufactured from these protein sources using *L. edodes* mycelia. The applicant did not carry out specific studies to determine the potential allergenicity of the NF but provided analytical data on the absence of the food allergens reported in Annex II of Regulation (EU) 1169/2011[6].

The applicant performed a literature search in order to retrieve relevant allergenicity data on rice, pea and Shiitake mushrooms. Allergic reactions to rice (Jeon et al., 2011) and peas (Sanchez-Monge et al., 2004) have been reported but are rare. In a systematic review by Nguyen et al. (2017), photosensitive skin lesions, contact dermatitis and systematic allergic contact dermatitis due to the consumption of raw Shiitake mushrooms were reported.

In conclusion, the NF contains protein from pea, rice and Shiitake mushroom (*L. edodes*), that have all been reported to trigger allergic reactions. The NF is therefore potentially allergenic upon oral consumption for pea-, rice- and Shiitake mushroom-allergic individuals. However, the Panel considers that this risk is expected not to be higher than that resulting from the normal consumption of pea, rice, or the fruiting body of the Shiitake mushroom (EFSA GMO Panel, 2021).

4. Discussion

The NF, which is the subject of the application, is pea and rice protein fermented by Shiitake (*L. edodes*) mycelia.

The applicant intends to market the NF as an ingredient in a number of food products. The Panel notes that the proposed uses of the NF include food categories that are generally not considered as protein sources.

[6] 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

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

Intake estimates for the NF consumed via foods in which it would be added as an ingredient were performed for the general population, based on the EFSA Comprehensive European Food Consumption Database. The highest intake estimate was calculated for infants at 3.7 g NF/kg bw per day at the 95th percentile.

The NF contains ≥ 75% protein which is well digestible and provides sufficient amounts of essential amino acids. The estimated P95 protein intakes from the NF exceed PRIs for all population groups (EFSA NDA Panel, 2012), and are 2.5- and 2.4-fold above the PRIs for infants, and young children.

Although a tolerable upper intake level (UL) has not been derived for protein (EFSA NDA Panel, 2012), the protein intake from the NF may nevertheless further contribute to an already high dietary protein intake in Europe (EFSA NDA Panel, 2012). The Panel also notes that the intake estimates are conservative and may overestimate the potential protein intake.

The different starting materials contained in the NF at the proposed uses are not considered to have any adverse or antinutritional effect on consumers.

No toxicological studies with the NF were provided by the applicant. The Panel considers that taking into account the nature of the NF and the history of safe use, no toxicological studies are required on the NF.

The NF contains protein from pea, rice and Shiitake mushroom (*L. edodes*), that have all been reported to trigger allergic reactions in sensitised individuals. The NF is therefore potentially allergenic upon oral consumption for pea-, rice- and Shiitake mushroom-allergic individuals. However, this risk is expected not to be higher than that resulting from the normal consumption of pea, rice or the fruiting body of the Shiitake mushroom.

5. Conclusions

The Panel concludes that the NF, pea and rice protein fermented by Shiitake (*Lentinula edodes)* mycelia, is safe under the proposed conditions of use.

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 data claimed as proprietary by the applicant (Detailed description of the production process, data concerning the composition and analysis of the novel food).

6. Steps taken by EFSA

1) On 22/04/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of pea and rice protein fermented by Shiitake mushroom (*Lentinula edodes*) mycelia as a novel food. Ref. Ares(2020)2177013 - 22/04/2020.
2) On 22/04/2020, a valid application on pea and rice protein fermented by Shiitake mushroom (*Lentinula edodes*) mycelia, which was submitted by MycoTechnology, Inc., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1459) and the scientific evaluation procedure was initiated.
3) On 28/07/2020 and 20/09/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 10/09/2021 and 10/01/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 28/02/2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of pea and rice protein fermented by Shiitake (*Lentinula edodes*) mycelia as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

AA	Amino acid
ADME	Absorption, distribution, metabolism and excretion
AID	Apparent Ileal Digestibility
AOAC	Association of Official Analytical Chemists
Bw	body weight
CFU	Colony Forming Unit
CP	crude protein
DIAAS	Digestibility Indispensable Amino Acid Scores
DW	dry weight
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
F	Fermented
FAO	Food and Agriculture Organization of the United Nations
EFSA	European Food Safety Authority
FDA	US Food And Drug Administration
FDA's BAM	FDA's Bacteriological Analytical Manual
GC-FID	Gas Chromatography with Flame ionisation Detector
GMP	Good Manufacturing Practice
GRAS	Generally Recognised as Safe
HACCP	Hazard Analysis Critical Control Points
ICP-MS	Inductively coupled plasma mass spectrometry
ITS	Internal Transcribed Spacer
IU	International Unit
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
NCBI	National Center for Biotechnology Information
NDA	Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
PDCAAS	Protein Digestibility Corrected Amino Acid Scores
PRI	Population Reference Intake
RH	relative humidity
SAA	sulfur amino acid
SCF	Scientific Committee on Food
SID	Standardised Ileal Digestibility
STTD	standardised total tract digestibility
TAMC	Total aerobic microbial count
TYMC	Total yeast and mould count
UF	Unfermented
UHPLC-MS/MS	Ultra high-performance liquid chromatography tandem mass spectrometry
UL	tolerable upper intake level

Appendix A – Batch-to-batch amino acid analysis

Amino acids (%) % Batch	#1	#2	#3	#4	#5
Indispensable AA					
Arg	6.25	6.30	6.35	6.51	6.38
His	1.77	1.81	1.83	1.89	1.84
Ile	3.61	3.61	3.71	3.67	3.71
Leu	6.48	6.42	6.60	6.54	6.65
Lys	4.39	4.37	4.52	4.61	4.52
Met	1.26	1.32	1.36	1.32	1.41
Phe	4.23	4.17	4.30	4.23	4.32
Thr	2.81	2.73	2.82	2.76	2.84
Trp	0.78	0.83	0.82	0.84	0.83
Val	4.29	4.32	4.41	4.36	4.42
Dispensable AA					
Ala	3.71	3.66	3.75	3.67	3.77
Asp	8.16	8.04	8.25	8.19	8.29
Cys	0.81	0.90	0.89	0.92	0.87
Glu	13.35	13.36	13.56	13.74	13.57
Gly	3.20	3.17	3.24	3.20	3.26
Pro	3.47	3.48	3.54	3.49	3.58
Ser	3.86	3.74	3.87	3.82	3.90
Tyr	3.15	3.17	3.26	3.23	3.29
Total AA	75.58	75.40	77.08	76.99	77.45

AA: amino acid.

Appendix B – Analysed amino acid composition of protein concentrate blends

Components (%)	UF	F
Dry matter	96.90	95.64
Crude protein	77.57	76.77
Indispensable AA		
Arg	6.60	6.34
His	1.95	1.87
Ile	3.84	3.63
Leu	6.54	6.39
Lys	5.53	3.97
Met	1.06	1.44
Phe	4.28	4.23
Thr	2.79	2.69
Trp	0.79	0.83
Val	4.33	4.49
Total	37.51	35.88
Dispensable AA		
Ala	3.51	3.77
Asp	8.28	7.62
Cys	0.85	1.06
Glu	12.64	12.78
Gly	3.13	3.18
Pro	3.27	3.37
Ser	3.34	3.24
Tyr	3.18	3.51
Total	38.10	38.43
Total AA	75.61	74.31

AA: amino acid.
UF: Unfermented blend 65% pea protein concentrate: 35% rice protein concentrate.
F: Fermented blend 65% pea protein concentrate: 35% rice protein concentrate.

Appendix C – Apparent ileal digestibility (AID) and standardised ileal digestibility (SID) of crude protein)

Apparent ileal digestibility (AID) and standardised ileal digestibility (SID) of crude protein (CP0 and amino acids (AA) in protein blends.†,‡

Item, %	AID				SID			
	UF	F	Pooled SEM	p-value	UF	F	Pooled SEM	p-value
Indispensable AA	71.4	81.3	4.84	0.167	85.3	94.8	4.84	0.187
Arg	88.7	91.2	2.09	0.400	94.4	97.6	2.09	0.308
His	79.9	87.1	4.11	0.235	85.9	93.6	4.11	0.203
Ile	80.7	87.6	4.16	0.261	85.9	93.6	4.11	0.238
Leu	81.0	88.1	4.06	0.236	85.9	93.3	4.06	0.215
Lys	84.4	88.3	3.30	0.424	89.9	95.0	3.30	0.298
Met	74.2	87.0	5.82	0.142	78.8	91.3	5.82	0.153
Phe	83.1	89.5	3.54	0.222	87.9	94.5	3.54	0.204
Thr	72.1	81.2	5.09	0.227	84.2	94.1	5.09	0.189
Trp	81.9	87.9	2.97	0.177	90.9	97.2	2.97	0.156
Val	76.9	85.4	4.61	0.212	83.6	92.3	4.61	0.203
Mean	81.5	87.8	3.73	0.255	87.5	94.3	3.73	0.222
Dispensable AA								
Ala	72.1	82.2	5.37	0.206	82.0	92.2	5.37	0.197
Asp	73.1	83.0	6.31	0.282	78.6	89.2	6.30	0.254
Cys	62.5	78.1	7.46	0.162	73.8	88.9	7.46	0.173
Glu	81.4	89.0	4.02	0.204	85.9	93.7	4.02	0.192
Gly	58.1	66.1	6.78	0.356	85.1	94.5	6.78	0.283
Ser	79.3	86.2	3.83	0.227	89.2	96.9	3.83	0.178
Tyr	81.8	88.5	3.66	0.218	87.2	94.0	3.66	0.209
Mean	70.2	77.6	5.30	0.339	77.6	85.5	5.30	0.313
Total AA	75.7	82.5	4.46	0.298	82.4	87.7	4.46	0.268

UF = Unfermented blend 65% pea protein concentrate: 35% rice protein concentrate.
F = Fermented blend 65% pea protein concentrate: 35% rice protein concentrate.
†: Data are least squares means of nine observations of unfermented (UF) and seven observations for fermented protein (F).
‡: Standardised ileal digestibility values were calculated by correcting values for apparent ileal digestibility for the basal ileal endogenous losses. Endogenous losses of CP and AA (g/kg dry matter intake) were as follows: CP, 19.55; Arg. 0.68; His, 0.21; Ile, 0.37; Leu, 0.60; Lys, 0.48; Met, 0.11; Phe, 0.38, Thr, 0.62; Trp, 0.13; Val, 0.55; Ala, 0.69; Asp, 0.85; Cys, 0.22; Glu, 1.11; Gly, 1.64; Ser, 0.63; Tyr, 0.28.

Appendix D – Undigestible carbohydrates and antinutrient content from starting materials, pea/rice blend and the NF

Parameter	Rice protein	Pea protein	Pea/rice blend	NF batch #7	NF batch #8
Phytic acid (%)	0.15	1.76	1.23	0.91	0.99
Trypsin inhibitors (TIU/g)	< 1,000	1,700	< 1,200	< 1,000	< 1,000
Chymotrypsin inhibitors μg/mL (IC50)	Not determined	325.3	390.1	246.9	259.2
Lectin (mg/g)	< 0.05	< 0.05	N/A	N/A	N/A
Oryzacystatin (IU/g)	6.583	< 0.00001	3.703	0.598	0.561
Rice alpha-amylase/subtilisin inhibitor (RASI) (IU/g)	94.06/0.047	15.11//0.00	54.59/0.00	31.25/0.00	18.99/0.00
Total polyphenols (mg/g)	< 2.00	< 2.00	< 2.00	2.00	< 2.00
Raffinose (g/kg)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Stachyose (g/kg)	< 0.5	< 0.5	< 0.5	0.9440	0.5830
Verbascose (g/kg)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

N/A: not analysed.

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.7205#support-information-section).