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Safety of partially hydrolysed protein from spent barley (*Hordeum vulgare*) and rice (*Oryza sativa*) 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 partially hydrolysed protein from spent barley (*Hordeum vulgare*) and rice (*Oryza sativa*) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is produced by enzymatically treating the pasteurised spent barley and rice residues of the mash step of beer production. Several mechanical treatment steps of the partial hydrolysate are employed to obtain the final product. The NF consists mainly of partially-hydrolysed proteins from spent barley and rice and it is in powdered form. The Panel notes that there are no safety concerns regarding the stability of the NF if the NF complies with the proposed specification limits during its entire shelf life of 15 months. The NF is proposed to be used as an ingredient in various foods such as dairy products, bakery products, pasta, fruit and vegetable spreads, snacks, and single meal replacements for weight reduction. The target population is the general population. Considering protein quality aspects, the Panel concludes that the NF is not a suitable protein source for the nutrition of infants. The Panel notes that, considering the composition of the NF, the proposed conditions of use and that the NF will not be the sole source of dietary protein, the consumption of the NF is not nutritionally disadvantageous. Taking into account the production process, the extensive compositional characterisation of the NF and the history of use of the source the Panel considers that no toxicological studies are required on the NF. Moreover, the Panel considers that the NF has the potential capacity to sensitise individuals and to induce allergic reactions in individuals allergic to barley and rice. With the exception of possible allergenicity, the Panel concludes that the NF is safe under the proposed uses and use levels.

Keywords: novel foods, food safety, *Hordeum vulgare*, *Oryza sativa*, barley, rice, food by-products

Requestor: European Commission

Question number: EFSA-Q-2020-00806

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1. Introduction

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

On 20 November 2020, the company Evergrain, LLC submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise the placing on the Union market of proteins obtained from barley (*Hordeum vulgare*) and rice (*Oryza sativa*) as a novel food. These proteins are produced by the concentration of proteins from a mixture of barley and rice using a series of enzymatic hydrolysis and mechanical purification steps.

The applicant requests to authorise the use of proteins obtained from barley (Hordeum vulgare) and rice (Oryza sativa) in a number of foods.

The applicant has also 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 proteins obtained from barley (*Hordeum vulgare*) and rice (*Oryza sativa*) as a novel food.

The European Commission asks the European Food Safety Authority to evaluate and inform the Commission as to whether and if so, to what extent, the requirements of Article 26(2)(c) of Regulation (EU) 2015/2283 are fulfilled in elaborating its opinion on proteins obtained from barley (*Hordeum vulgare*) and rice (*Oryza sativa*) regarding the proprietary data for which the applicant is requesting data protection.

1.2. Additional information

On 18 May 2022, the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) adopted a scientific opinion on the safety of the food enzyme pullulanase from the genetically modified *Bacillus licheniformis* strain NZYM-LU (EFSA CEP Panel, 2022a). On 19 May 2022, the EFSA CEP Panel adopted a scientific opinion on the safety of the food enzyme glucan 1,4-α-glucosidase from the genetically modified *Aspergillus niger* strain NZYM-BE (EFSA CEP Panel, 2022b). Based on the data provided, the Panel concluded that both aforementioned food enzymes do not give rise to safety concerns under the intended conditions of use.

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 requests 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 for 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, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise a report on the protein quality of the NF, information on the inactivation of the enzyme in the NF, information on the absence of toxigenic potential in the enzyme preparation, information on the absence of mycotoxins and other secondary metabolites produced by *A. niger* in the enzyme preparation, compositional data (certificates of analyses of the NF batches) and the stability study report.

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, pp. 1–22.

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

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.

Additional information which was not included in the application was retrieved by literature search following a search strategy and standard operating procedure as described by Dibusz and Vejvodova (2020).

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

3. Assessment

3.1. Introduction

The NF, which is the subject of the application is partially hydrolysed proteins from spent barley (*Hordeum vulgare*) and rice (*Oryza sativa*) obtained from beer mash, the solid by-product of beer production.

The NF, as described in Article 3(2)(v) of Regulation (EU) 2015/2283, falls under the category 'food consisting of, isolated from or produced from plants or their parts, except when the food has a history of safe food use within the Union and is consisting of, isolated from or produced from a plant or a variety of the same species obtained by:

- traditional propagating practices which have been used for food production within the Union before 15 May 1997; or
- non-traditional propagating practices which have not been used for food production within the Union before 15 May 1997, where those practices do not give rise to significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances'.

The NF is produced by enzymatically treating the pasteurised spent barley and rice residues of the mash step of beer production. Several mechanical treatment steps of the partial hydrolysate are employed to obtain the final product. The NF consists mainly of partially-hydrolysed proteins from spent barley and rice. The NF is proposed to be used, in the form of powder, as an ingredient in various food products such as dairy products, bakery products, pasta, fruit and vegetable spreads, snacks, and single meal replacements for weight reduction. Food products containing the NF can be consumed by the general population, as well as the single meal replacements.

3.2. Identity of the NF

The NF is partially hydrolysed protein from spent barley (*H. vulgare*) and rice (*O. sativa*), residues obtained from the solid by-product of beer production that contains 45–70% spent barley and 30–55% spent rice.

H. vulgare (Poaceae) has the common names 'barley' and 'common barley'. *O. sativa* (Poaceae) has the common name 'rice'. The parts used for the production of the NF are the spent grains/seeds of barley and the seeds of rice after their use in beer production.

The applicant was requested to characterise the protein fraction of the NF. At least five batches of the minimum and maximum barley-to-rice ratio were analysed for the degree of protein hydrolysis (OPA assay, Nielsen et al., 2001) and the molecular weight distribution (size exclusion-high-performance liquid chromatography, HPLC-SEC). The protein hydrolysis was between 1.11% and 5.55%, while the main molecular weight distribution was within the range of 0.5–30 kDa.

The applicant states that the raw materials used for the extraction of the proteins are cultivated in North America and that the spent barley and rice grains used as raw materials are not from genetically modified plants. The NF is intended to be marketed as a powder.

Moreover, the applicant measured the degree of hydrolysis of the NF in several batches of the NF (six for a barley-to-rice ratio of 70:30, three for a barley-to-rice ratio of 60:40 and five for a barley-to-rice ratio of 45:55). The Panel notes that the degree of hydrolysis is not consistent neither among NF

batches in general, nor for batches of the same barley-to-rice ratio. The values vary from 1.11% to 5.55% (Table 1). The applicant informed EFSA that the design of the production process (Section 3.3, Production Process) enables the removal of most low molecular weight components from the NF. The targeted degree of hydrolysis is below 7%.

Table 1: Degree of hydrolysis of proteins in the NF

Parameter (unit)	Batch number													
Barley to rice ratio	70:30[(a)]						60:40[(a)]			45:55[(a)]				
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14
Protein degree of hydrolysis (%)	4.29	1.11	4.31	2.18	2.24	3.51	1.63	3.51	1.87	2.28	2.49	5.55	5.46	4.1

NF: novel food.
(a): Analytical method: OPA assay (Nielsen et al., 2001).

To further characterise the identity of the NF, the applicant provided data on the molecular weight (MW) distribution of the NF. For 8 out of 14 batches tested, around 80% of the molecules had a MW between 1 and 30 kDa, whereas, for the remaining six batches, 80% of the molecules had a MW between 0.5 and 10 kDa. The MW does not appear to be dependent on the barley-to-rice ratio used. The in-house analytical method employed was based on the method described by Visser et al. (1992). HPLC-SEC was used with UV (220 and 280 nm) and refractive index detection (UV-RID). Data on the validation of the method have been provided (Table 2).

Table 2: Molecular weight distribution of the NF

Barley to rice ratio	Batch number													
	70:30[(a)]						60:40[(a)]			45:55[(a)]				
Parameter (%)	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14
> 66,000 Da	1.8	3.1	2	2.6	2.9	1.3	3.7	2.8	3.2	3.6	2	1	0.9	0.8
30,000 to 66,000 Da	2.6	3	2.4	3.1	2.4	1.8	4.1	3	3.3	3.3	2.2	2.2	2.2	2.3
10,000 to 30,000 Da	9.0	12.2	9.5	12.1	11	9	12.8	13.7	12.8	12	11.3	8.5	8.5	10.9
5000 to 10,000 Da	14.5	16.8	14.3	16.3	16.2	14.1	16.5	15.7	16.6	15.9	16	11	11	13.1
3,000 to 5,000 Da	12.5	16.9	11.7	16.6	17.3	15.1	14.6	15.2	16.3	16.7	17.4	10.7	10.6	13
2,000 to 3,000 Da	13.4	14	13.5	13.9	14.6	13.2	12.8	12.7	13.6	14	14.8	10.6	10.3	12
1,000 to 2,000 Da	21.8	19.3	22.3	19.3	20.3	23.2	18.5	20.7	19	19.3	20.6	23.6	23.1	21.5
500 to 1,000 Da	16.5	9.6	16.3	10.4	10.2	15.2	11.6	11	9.7	9.8	10.6	20.9	21.1	16.6
0 to 500 Da	8.1	5.1	8.1	5.8	5.1	7.3	5.3	5.1	5.5	5.4	5	11.5	12.2	9.9

(a): Analytical method: Size-exclusion high-performance liquid chromatography ultraviolet refractive index detection (HPLC-SEC UV-RID), Visser et al. (1992).

3.3. Production process

According to the information provided, the NF is produced in line with Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles, under the British Retail Consortium (BRC) and the Food Safety System Certification (FSSC) 22000 certification.

In the first step of the manufacturing process, pasteurised spent grains from malted barley and rice obtained from the mash step of beer production are added to a hydrolysis reactor tank with water in a pre-defined ratio. The slurry is mixed, under agitation, at temperatures exceeding 68°C, and under atmospheric pressure, for up to 3 h. The grain mixture is then brought to a specific temperature and treated with a mixture of glucoamylase and pullulanase obtained from two genetically modified

A. niger and *B. licheniformis* strains, respectively, for 45 min to hydrolyse starch. The pH of the solution is raised to 9.0. The mixture is then treated with a serine protease obtained from a genetically modified *B. licheniformis* strain to hydrolyse the protein component. The enzymes are deactivated by heating.

To prove the deactivation of the enzymes, the applicant provided additional information on the residual enzyme activity in several batches of the NF of different barley-to-rice ratios.

For all three enzymes used (glucoamylase, pullulanase, serine protease), the absence of recombinant DNA from the production strains in the enzyme preparations was demonstrated in accordance with the requirements in the EFSA Scientific Guidance for the submission of dossiers on Food Enzymes (EFSA CEP Panel, 2021). Three independent production batches of each enzyme were analysed in triplicate by polymerase chain reaction (PCR), targeting a fragment specific to each of the production strains. The levels of recombinant DNA were below the limit of detection (LOD) of 10 ng DNA/g product in all cases. The absence of viable cells of the production microorganisms was also demonstrated in three independent production batches of each of the enzymes. The analysis was performed in triplicate.

In the second step, the solids are separated from the liquid protein stream by pumping the slurry from the extraction tank to decanting centrifuges. The decanted solids are then mixed with water and decanted again to increase protein recovery. The washed and decanted solids are pushed through a screw press, as needed, to further increase protein recovery and reduce the moisture content. The liquid from the hydrolysis is fed into a microfiltration system (0.1 μm membranes at 70 to 80°C) with multiple loops of diafiltration to increase protein recovery in the permeate. The retentate contains fat, fibre, and large protein molecules. Following the microfiltration step, the permeate from the microfiltration is processed in a nanofiltration system (*ca*. 600 to 1,000 Da cut-off). The retentate of the nanofiltration is kept and comprises around 20% solids, of which over 85% is crude protein. The next step involves vacuum evaporation to remove water from the product, which raises the solid concentration of the retentate from 20% to 50%. The output is then pasteurised and sent to the spray dryer to obtain powdered protein, which is packaged in lined, sealed kraft paper bags or sealed containers.

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with the required characteristics, the applicant provided analytical information for several independent batches of the NF (Table 3).

Table 3: Proximate analysis of the NF

Parameter (unit)	Batch number															Analytical method
Barley to rice ratio	70:30							60:40			45:55					
	#5	#15	#16	#6	#17	#18	#19	#20	#21	#22	#12	#13	#14	#23	#10	#11	
Protein (g/100 g of NF)	82.7	84.4	88.8	82.4	86.1	85.8	83	81.6	89.3	89.6	86.2	84.8	86.8	87.2	83.7	84.2	Dumas, AOAC 990.03, AOAC 992.15
Crude fat (g/100 g of NF)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	< 0.10	0.12	NR	NR	Acid hydrolysis, AOAC 954.02
Total carbohydrates (g/100 g of NF)	8.08	8.97	3.12	8.56	< 0.01	4.58	7.95	9.31	3	3.56	5.16	6.6	< 0.5	NR	7.05	5.47	Calculation by difference, CFR 21
Of which sugars (g/100 g of NF)	< 0.35	0.37	< 0.35	0.83	0.72	0.76	< 0.35	< 0.35	0.82	0.47	1.27	1.53	0.98	NR	0.49	< 0.35	LC, AOAC 982.14, modified
Dietary fibre (g/100 g of NF)	3.2	3.7	2.2	2.3	2.2	2.5	4.1	2.5	2	2.7	2.2	1.9	NR	NR	3.4	3.6	Enzymatic, gravimetric, AOAC 991.43
Moisture (g/100 g of NF)	4.2	5.3	3	4	4.1	4.3	5.3	4.7	3	2.8	2.9	2.9	NR	3.9	4.7	4.5	Gravimetric, AOAC 925.09
Ash (g/100 g of NF)	4.78	1.15	4.94	4.76	5.06	5.19	3.47	3.99	4.69	4.08	5.53	5.57	NR	5.22	4.34	5.61	Gravimetric, AOAC 942.05
Energy (kcal/ 100 g of NF)	365	375	369	366	364	363	366	367	369	372	367	367	NR	NR	365	361	Regulation (EU) 1169/2011

AOAC: Association of Official Analytical Chemists; NR: not reported; CFR: Code of Federal Regulations; EU: European Union; LC: liquid Chromatography.

Initially, the applicant provided analytical data on the levels of mycotoxins for several batches of the NF of different barley-to-rice ratios. The Panel, noting the magnitude of the LODs of the analytical methods implemented (aflatoxins: < 5 μg/kg, ochratoxin A: < 5 μg/kg), requested the applicant to repeat the analysis using methods with lower LODs. The applicant provided analytical data on the levels of aflatoxins (B1, B2, G1, G2), ochratoxin A, fumonisins (B1, B2), deoxynivalenol, toxin HT2, toxin T2 and zearalenone in the NF (Table 4). The values reported are below the limit of quantitation (LOQ) of the analytical methods implemented. The LOQ values are lower than the maximum levels (MLs) set for foodstuffs in Regulation (EC) No 1881/2006[3].

Table 4: Mycotoxins in the NF

Parameter (μg/kg)	**Batch number**											**Analytical method**
Barley to rice ratio	**70:30**					**60:40**	**45:55**					
	#5	**#19**	**#16**	**#24**	**#25**	**#26**	**#14**	**#12**	**#13**	**#10**	**#11**	
Aflatoxins B1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	DIN EN 14123 (2008-03), mod.
Aflatoxins B2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins G1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	
Aflatoxins G2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 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	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	
Ochratoxin A	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	
Fumonisins (Sum of B1, B2)	< 30	53	< 30	< 40	< 40	< 30	< 30	< 30	< 30	< 30	< 30	LC–MS/MS, J AOAC, 92 (2), 496
Deoxynivalenol	< 10	< 10	< 10	< 20	< 20	< 10	< 50	< 50	< 50	< 50	< 50	LC–MS/MS, Food Addit Contam Part A, 2013:303), 541–9
Toxin HT2	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	
Toxin T2	< 1	< 1	< 1	< 10	< 10	< 1	< 1	< 1	< 1	< 1	< 1	
Zearalenone	< 5	< 5	< 5	< 10	< 10	< 5	< 5	< 5	< 5	< 5	< 5	

LC–MS/MS: liquid chromatography–tandem mass spectrometry; DIN: Deutsche Institut für Normung (German Institute for Standardization); EN: Europaische Norm (European standards); AOAC: Association of Official Analytical Chemists.

Concentrations of heavy metals in the NF analysed by inductively coupled plasma mass spectrometry (ICP-MS) are reported in Table 5. The applicant compared the values to the MLs for other foods as set in Regulation (EC) No 1881/2006. Regarding arsenic, the concentration for the analysed NF batches ranged from 13 up to 177 μg/kg (0.013–0.177 mg/kg). The Panel notes that these values are below the currently established MLs of arsenic in other foods (non-parboiled milled rice (polished or white rice): 0.20 mg/kg; parboiled rice and husked rice: 0.25 mg/kg; rice waffles, rice wafers, rice crackers and rice cakes: 0.30 mg/kg). Moreover, the highest cadmium concentration observed among the analysed NF batches (0.034 mg/kg) is almost five times lower than the established ML of 0.15 mg/kg for rice, and lower than the established ML of 0.05 mg/kg for barley. Lead and mercury were below the LOQ in all the NF batches analysed.

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

Table 5: Heavy metals in NF

Parameter	Batch number														Analytical method
Barley to rice ratio	70:30						60:40			45:55					
	#19	#5	#15	#16	#25	#24	#22	#20	#21	#14	#23	#10	#12	#11	
Arsenic (µg/kg)	36.5	24.4	71.0	13.0	110.0	31.0	13.0	49.0	18.0	176.0	177.0	29.9	63.0	29.7	ICP-MS, J AOAC vol 90 (2007) 844–856 modified
Cadmium (µg/kg)	23.7	5.5	13.0	24.0	18.0	26.0	24.0	16.0	24.0	< 10	13.0	20.4	14.0	13.4	
Lead (µg/kg)	< 5	< 5	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 5	< 10	< 5	
Mercury (µg/kg)	< 5	< 5	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 5	< 10	< 5	

ICP-MS: inductively coupled plasma mass spectrometry; AOAC: Association of Official Analytical Chemists.

Analytical data of the pesticide residue concentrations for seven independently produced batches of NF have been provided. The results showed that the analysed pesticide residue concentrations in the NF are below the LOD or LOQ of the analytical method used (GC–MS/MS and LC–MS/MS, AOAC 2007.01).

The applicant provided water activity values (Table 6) and microbiological data (Table 7) on several independently produced batches of the NF.

Table 6: Water activity of the NF

Parameter	Batch number												Analytical method
Barley to rice ratio	70:30					60:40		45:55					
	#27	#28	#29	#10	#19	#30	#31	#10	#11	#12	#13	#14	
Water activity (aw)	0.134	0.131	0.275	0.188	0.163	0.327	0.255	0.227	0.215	0.221	0.220	0.180	Internal, Hygrometry (dewpoint)

Table 7: Batch-to-batch microbiological analysis of the NF

Parameter (unit)	Batch number													Analytical method
Barley to rice ratio	70:30						60:40		45:55					
	#5	#16	#19	#6	#17	#18	#22	#21	#12	#13	#14	#23	#10	
Total (Aerobic) Plate Count (CFU/g)	< 100	70	400	< 10	< 10	3400	< 10	240	14000	750	12000	< 10	800	AOAC 966.23
Coliforms (CFU/g)	< 100	< 10	< 100	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 100	AOAC 991.14
Enterobacteriaceae (CFU/g)	< 100	/	< 100	/	/	/	/	/	/	/	/	/	< 100	AOAC 2003.01
***Escherichia coli* (CFU/g)**	< 100	< 10	< 100	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 100	AOAC 991.14
Yeasts (CFU/g)	< 10	< 10	< 100	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	AOAC 2014.05
Moulds (CFU/g)	10	< 10	< 100	< 10	< 10	< 10	< 10	< 10	20	30	< 10	30	< 10	AOAC 2014.05
***Listeria* spp. (in 25 g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	BAM Chapter 18
***Staphylococcus aureus* (CFU/g)**	< 100	< 10	< 100	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 100	AOAC 2003.07
***Salmonella* spp. (in 25 g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	AOAC-RI 121501
***Bacillus cereus* (CFU/g)**	< 10	< 10	< 100	< 10	< 10	< 10	< 10	< 10	< 10	10	56	< 10	< 10	AOAC 980.31

CFU: colony forming units; AOAC: Association of Official Analytical Chemists; BAM: Bacteriological Analytical Manual; ND: not detected; /: not provided.

The applicant analysed the free amino acid profile in 10 batches of the NF, five per extreme barley-to-rice ratios (70:30 and 45:55) (EN ISO 13903:2005, ion chromatography coupled to spectrophotometric detection (IC-UV)). Free glutamic acid (~ 1 g/kg) and free leucine (~ 0.7 and ~ 1.3 g/kg) were quantified in two batches, free alanine in one batch (~ 0.6 g/kg) and free phosphoserine in five batches (~ 6.5 g/kg). The values of the rest of the free amino acids, in the remaining batches, were below the LOQ (0.5 g/kg) of the analytical method implemented (EN ISO 13903:2005 mod.). The nanofiltration step (Section 3.3, Production process), removes the free amino acids. Further quantitative information on the free amino acid profile of the NF is provided in Appendix A.

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 NF is packaged in kraft paper bags (approximately 20 kg per bag) and stored in dry conditions at ambient temperature. The proposed shelf life of the NF is 15 months.

The applicant monitored the microbiological stability of the NF by monitoring several independently produced batches of the NF during storage (Table 8). The NF batches were stored at normal storage conditions at 15–29°C and at 30–75% of relative humidity (RH) for a period of up to 15 months.

The Panel notes the variability regarding the aerobic plate counts observed among the various NF batches analysed. Upon EFSA's request, the applicant clarified that the NF batches with higher aerobic plate counts were batches produced relatively early in the manufacturing attempts of the NF and since then, the automation of certain steps allowed for improving the microbiological profile of the NF (lower total aerobic count (TAC) values).

Since the NF is going to be used as an ingredient of other food products, EFSA asked the applicant to investigate the stability when the NF is used as an ingredient in the intended-for-use matrices. The applicant investigated the microbiological profile of a 'Ready to Drink Chocolate Milk' containing the NF as an ingredient (2.27% incorporation level, 60:40 barley-to-rice ratio) after manufacture and after 6 months of storage. The counts of total aerobic plate counts, coliforms, *E. coli*, and *Salmonella* spp., were reported < 10 CFU/g, < 10 CFU/g, < 3 MPN/g and not detected, respectively (both at t = 0 months and t = 6 months).

In addition, the applicant was asked to provide data on the formation of processing contaminants in a food matrix produced using a process that includes heat treatment. The applicant provided analytical data on acrylamide (LC–MS/MS) and furan (2-methylfuran, 3-methylfuran, 2,5-dimethylfuran) (HS-GC–MS) concentrations in brownies and plant-based beverages containing the NF in two different ratios (70:30 and 45:55). Products containing pea protein instead of the NF were used as control samples. In the brownies containing the NF, the acrylamide concentrations were reported to be 11 and 14 μg/kg (for 70:30 and 45:55 barley-to-rice ratios, respectively), whereas the acrylamide concentration in the brownie with pea protein was reported to be 6.5 μg/kg. In all the beverage samples tested, the concentration of acrylamide was reported to be < 5 μg/kg. All furans tested, in all samples, were reported to be < 10 μg/kg.

The Panel notes that the microbiological values of most of the analysed NF batches do not exceed the given specifications. However, two batches exceed the specification limit for TACs (Tables 7 and 8). Additionally, the Panel notes that the analytical data regarding the putative formation of contaminants due to the use of NF as an ingredient in the intended-for-use matrices are limited due to the number of samples tested (one per formulation). The Panel also notes that the food items containing the NF have to comply with existing legislative limits, such as the benchmark levels of acrylamide in bakery products established by Regulation (EU) 2017/2158[4]. 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 on process-formed contaminants, the stability data do not raise safety concerns.

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

Table 8: Microbiological profile status of the NF during storage

Parameter (unit)	Batch number																	
Barley-to-rice ratio	**70:30**					**45:55**				**70:30**					**45:55**			
Batch number	**#32**	**#16**	**#1**	**#3**	**#6**	**#12**	**#13**	**#33**	**#34**	**#32**	**#16**	**#1**	**#3**	**#6**	**#12**	**#13**	**#33**	**#34**
Time (months)	**0**									**13**	**14**	**15**						
Total (aerobic) plate count (CFU/g)	30	70 (est)	3400	< 10	< 10	14000	750	530	2600	< 100	200 (est)	5000 (est)	< 100	200 (est)	14000	3600	100 (est)	6700
Coliforms (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts and moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Salmonella* spp. (per 25 g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Escherichia coli* (CFU/g)**	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Coagulase-positive *Staphylococcus* (CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
***Listeria monocytogenes* (per 25 g)**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
***Bacillus cereus* (CFU/g)**	< 10	< 10	< 10	< 10	< 10	10	< 10	< 10	10	< 10	40	< 10	< 10	< 10	60	< 10	< 10	< 10

NF: novel food; CFU: colony forming units; ND: not detected; est: estimated.

3.5. Specifications

The specifications of the NF are indicated in Table 9.

Table 9: Specifications of the NF

Description: Partially hydrolysed protein from spent barley and rice	
Source: Spent Barley (*Hordeum vulgare*) (45–70%) and spent rice (*Oryza sativa*) (30–55%)	
Parameter	**Specification**
Organoleptic	
Taste	Malty, cocoa
Colour	Brown
Appearance	Powder
Odour	Malty, roasted
Degree of hydrolysis	1–7%
Proximate parameters	
Protein (N × 6.25)	78–90%
Moisture	2–8%
Carbohydrates	2–10%
Fat	0–2%
Ash	1–8%
Heavy metals	
Arsenic	< 0.2 mg/kg
Cadmium	< 0.1 mg/kg
Lead	< 0.2 mg/kg
Mercury	< 0.1 mg/kg
Mycotoxins	
Aflatoxin B1	≤ 2 μg/kg
Sum of aflatoxins (B1, B2, G1, G2)	≤ 4 μg/kg
Deoxynivalenol	< 200 μg/kg
Fumonisins (sum of B1, B2)	≤ 200 μg/kg
Ochratoxin A	≤ 3 μg/kg
Zearalenone	≤ 20 μg/kg
Patulin	< 50 μg/kg
Antinutritional factors	
Phytic acid	< 0.25%
Microbiological	
Total aerobic plate count	$< 10^4$ CFU/g
Coliforms	< 100 CFU/g
Yeasts and moulds	< 100 CFU/g
Salmonella spp.	Not detected in 25 g
Escherichia coli	< 10 CFU/g
Staphylococcus aureus	< 10 CFU/g
Listeria monocytogenes	Not detected in 25 g
Bacillus cereus	< 100 CFU/g

NF: novel food; CFU: colony forming units.

The Panel is of the view that the specification limit for TAC of $< 3 \times 10^4$ CFU/g initially proposed by the applicant should be reduced to 10^4 CFU/g, considering the respective counts resulted in the batch-to-batch analysis, as well as the applicant's comment on the automation of certain steps of the production process that allowed for improving the microbiological profile of the NF (lower TAC values). The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

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

There is no history of use of the NF in the EU. However, the NF is a protein hydrolysate obtained from beer mash, the by-product of beer production, composed of spent barley (*H. vulgare*) and rice (*O. sativa*), both of which have a recognised history of consumption in the EU. The beer mash, also mentioned as brewers' spent grain, is mostly used as animal feed (Ikram et al., 2017).

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 to be used as an ingredient in several food products. These food products, defined using the FoodEx2 hierarchy, and the maximum use levels, are reported in Table 10.

Table 10: Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max use level (g/100 g)
L2	A0EZX	Fried or extruded cereal, seed or root-based products	5
L2	A04PE	Confectionery including chocolate	5
L3	A00EJ	Muesli and similar mixed breakfast cereals	5
L3	A040M	Pastas and rice (or other cereal)-based dishes	8
L3	A0B9J	Soups (dry mixture uncooked)	50
L3	A041L	Soups (ready-to-eat)	5
L3	A043Q	Gravy Ingredients	10
L3	A16GK	Savoury sauce dry preparation	50
L3	A03TE	Meat imitates	15
L3	A00EY	Cereal bars	30
L4	A039F	Butter and margarine/oil blends	10
L4	A02QB	Ice cream, milk-imitate based	10
L4	A03TH	Milk imitates	5
L4	A0F0M	Nut/seeds paste/emulsion/mass	15
L4	A03GA	Energy drinks	8
L4	A03GB	Isotonic and sport drinks	5
L4	A03FQ	Cola type drinks	5
L4	A03GF	Powdered drink bases	90
L4	A03DH	Multivitamin juices	5
L4	A03RV	Single meal replacement for weight reduction	30
L5	A03TR	Imitation cream	10
L5	A03TY	Imitation cheese	10
L5	A03TZ	Imitation yoghurt, non-soy	10
L5	A03VN	Hummus	10
L5	A03MG	Beer, alcohol-free	5

3.7.3. Anticipated intake of the NF

EFSA assessed the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 10), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile (P95) anticipated daily intake of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 11. The lowest and highest mean and P95 anticipated daily intake of the NF (on a mg/day basis), among the EU dietary surveys, are presented in Table 12.

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, Annex A).

Table 11: Intake estimate (mg/kg bw per day) resulting from the use of the NF 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	0.58	52.97	0.00	364.35
Young children[(c)]	1 to < 3	41.87	143.36	105.21	576.92
Other children	3 to < 10	45.29	343.53	228.98	902.78
Adolescents	10 to < 18	37.65	196.30	199.49	738.79
Adults[(d)]	≥ 18	4.25	200.65	20.50	785.90

bw: body weight; NF: novel food.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 6/4/2023. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 6/4/2023. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 based on less than 60 individuals are not considered).
(c): Referred to as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant, and lactating women.

Table 12: Intake estimate (mg/day) resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels

Population group	Age (years)	Mean intake (mg/day)		P95 intake (mg/day)	
		Lowest[(a)]	Highest[(a)]	Lowest[(b)]	Highest[(b)]
Infants	< 1	6	472	0	3,000
Young children[(c)]	1 to < 3	432	1,841	1,363	6,756
Other children	3 to < 10	1,133	8,554	5,725	20,563
Adolescents	10 to < 18	1,958	11,917	10,500	41,933
Adults[(d)]	≥ 18	299	13,205	1,667	50,587

NF: novel food.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 6/4/2023. The lowest and the highest averages observed among all EU surveys are reported in these columns.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 6/4/2023. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 based on less than 60 individuals are not considered).
(c): Referred to as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant, and lactating women.

3.7.4. Estimate of exposure to undesirable substances

Based on the highest P95 intake estimate (Table 11), EFSA estimated exposure to undesirable substances (heavy metals, toxins) from the NF, for all population groups. The specification limits (Table 9) were used as the maximum concentrations of the undesirable substances. When specification limits for a substance of possible concern have not been proposed, the maximum values reported for the analysed batches were used. The Panel considers that the consumption of the NF under the proposed uses and use levels does not contribute substantially to the overall intake of the analysed heavy metals, mycotoxins, and other undesirable substances through diet. The possibility of a high intake of manganese (Mn) from the NF is addressed in Section 3.9, Nutritional information.

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

No ADME data have been provided for the NF. A literature review on *in vitro* digestibility of rice and barley showed that they would be absorbed and handled by the body in normal metabolic processes, similar to that of other dietary protein sources (Hamaker et al., 1987) (FAO, 1999) (Cervantes-Pahm et al., 2014).

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF, which consists mainly of crude protein (85.4 ± 2.5%), carbohydrates (5.8 ± 2.7%) and ash (4.5 ± 1.1%). The energy value of this NF on average is 367 kcal/100 g of NF. Analytical data on the amino acid composition (Appendix B), minerals and vitamins (Tables 13 and 14), and antinutritional factors (Appendix C) have been provided for several batches of the NF. The fatty acid content was also investigated by the applicant (< 2%).

Protein content and protein quality

The NF contains on average 85.4 ± (2.5) g crude protein per 100 g, measured by the Kjeldahl method and calculated using a nitrogen-to-protein conversion factor of 6.25. The applicant quantified the amino acids of the protein in several batches of the NF according to AOAC 982.30 and AOAC 988.15 and all indispensable amino acids (IAA) were found to be present.

In addition, the applicant investigated the true ileal digestibility using a dynamic computer-controlled *in vitro* model of the stomach and small intestine (tiny-TIM) (Unpublished study report, 2022). The NF sample tested was produced usinga barley-to-rice ratio of 70:30%. Whey protein was used as a reference protein, while six more proteins of plant nature were analysed for comparison [two pea protein isolates, a mixture of the NF with pea protein (45:55%), soy protein, brown rice protein, hydrolysed wheat gluten]. The tests were conducted by an accredited laboratory in accordance with Good Laboratory Practice (GLP). The nitrogen digestibility was expressed as a percentage of the total nitrogen intake, including non-protein nitrogen. The true ileal digestibility was higher for whey protein (94.9 ± 0.5%) compared to the NF (90.1 ± 2.3%), indicating that the protein in the NF is less bio-accessible than whey protein. The hydrolysed wheat gluten had the highest true ileal digestibility (110.4 ± 6.2%), followed by one of the pea protein isolates (98.6 ± 2.2%), while the other pea protein isolate had a true ileal digestibility between those of whey protein and the NF (93.6 ± 0.9%). The other three test materials had true ileal digestibility values lower than the one of the NF. Following the recommendation by the Food and Agriculture Organisation of the United Nations (FAO) (FAO, 2013), the protein quality was determined by the 'Digestible Indispensable Amino Acid Score (DIAAS)'. For this, the true ileal digestibility of individual indispensable amino acids was also determined. Based on the FAO-recommended amino acid scoring patterns (FAO, 2013), the digestible IAA reference ratios for each indispensable amino acid were calculated. The resulting DIAAS for the NF corresponded to 45% for infants up to 6 months, 55% for children 6 months to 3 years, and 65% for other children, adolescents, and adults. The first limiting amino acid for the NF was lysine, for all groups. In comparison, DIAAS for whey protein was 94% and 75% for the mixture of the NF with pea protein, while DIAAS values for the other test products ranged between 36% and 63%. The Panel notes that according to FAO (2013), a DIAAS of 75% and higher indicates a protein of good quality. Considering the quality of the protein, the NF is not a suitable protein source for the nutrition of infants. If the NF entirely replaces other protein sources of higher quality, it might negatively impact protein nutrition also of the other age groups in cases when the overall protein intake is low. Based on the highest P95 intake levels of the NF (Section 3.7.3, Table 11) with a maximum protein content of 90% (Section 3.5, Table 9), the corresponding protein intake per kg bw per day from the NF would amount up to 0.33 g for infants, 0.52 g for young children, 0.81 g for other children, 0.67 for adolescents and 0.71 g for adults. These intakes correspond to up to 54%, 96%, 80%, and 85% of the respective population reference intake for protein for young children, other children, adolescents, and adults, respectively (EFSA NDA Panel, 2012). Provided that the NF would not be the sole source of dietary protein, that it is integrated into a varied and mixed diet, and considering that the average protein intake in the EU population is high and frequently above the dietary reference values (DRVs) (EFSA NDA Panel, 2012), the consumption of the NF is not expected to negatively impact protein nutrition.

Fatty acids, vitamins, and minerals

The NF contains a low amount of fat (< 2%). The applicant provided analytical data on the levels of some minerals and vitamins (Tables 13 and 14).

Considering the higher concentrations reported in Tables 13 and 14 and the estimated P95 of exposure to the NF, the Panel notes that none of the existing upper levels for the analysed micronutrients are expected to be exceeded from the NF alone, for any population group.

Intake of Mn, for which upper levels have not been established by EFSA, was also considered. The Scientific Committee on Food (SCF) (SCF, 2000) reported that exposure to high levels of Mn by

inhalation or oral intake may be neurotoxic. The SCF could, however, not set a tolerable upper intake level (UL) for Mn and concluded that '*the margin between oral effect levels in humans, as well as experimental animals and the estimated intake from food is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to Mn beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit*' (SCF/NDA, 2006).

The concentration of Mn in the NF according to the highest value observed among the analysed NF batches (Tables 13 and 14), may reach 2.1 mg/kg. This concentration is considerably lower when compared to food sources rich in Mn, e.g. nuts 24.9 mg/kg; dried fruit, nuts, and seeds 11.9 mg/kg; chocolate 8.9 mg/kg; bread, miscellaneous cereals 8.0 mg/kg (EFSA NDA Panel, 2013). EFSA estimated the intake of Mn from the NF, considering the highest value observed among the analysed NF batches (Tables 13 and 14) and the estimated daily intake of the NF for all population groups (Table 12).

EFSA has previously reported that estimated mean Mn intakes for adults in the EU ranged from 2 to 6 mg/day, with the majority of values being around 3 mg/day (EFSA NDA Panel, 2013). In younger age groups, mean Mn intakes in various EU countries ranged from 1.5 to 3.5 mg/day in children and from 2 to 6 mg/day in adolescents (EFSA NDA Panel, 2013). The highest estimated P95 intake of Mn from the NF ranges from 0.004 mg/day in infants to 0.12 mg/day in adults. As compared to the highest mean background Mn intake estimates, the additional intake of Mn from the NF would be 0.41% for young children, 1.23% for other children, 1.47% for adolescents, and 1.77% for adults. The Panel considers that such an increase in Mn intake (< 5% of the highest mean background intake[5]) from the NF is not of concern.

Table 13: Minerals and vitamins in the NF with a barley-to-rice ratio – 70:30

Parameter (unit)	Batch number (barley-to-rice ratio = 70:30)					Analytical method
	#5	#24	#19	#25	#35	
Iron (mg/100 g)	1.4	1.1	2.2	0.7	0.8	ICP, AOAC 984.27 mod, 927.02 mod, 985.01 mod, 965.17 mod
Potassium (mg/100 g)	828	1090	738	1060	937	
Sodium (mg/100 g)	1200	1090	1230	1060	937	
Calcium (mg/100 g)	30	28	23	29	31	
Magnesium (mg/100 g)	71	94	85	96	97	
Phosphorus (mg/100 g)	120	110	100	120	130	
Copper (mg/100 g)	0.36	0.75	0.89	0.60	0.56	
Manganese (mg/100 g)	0.12	0.14	0.21	0.14	0.14	
Zinc (mg/100 g)	0.50	0.72	0.66	0.79	0.70	
Silicon (mg/100 g)	41	43	30	60	41	
Sulphur (mg/100 g)	860	990	1,000	1,000	940	
Vitamin D2 (IU/100 g)	< 4	< 4	< 4	< 4	< 4	LC–MS/MS, Huang et al., Rapid Commun. Mass Spectrum 2014, 28
Vitamin D3 (IU/100 g)	< 4	< 4	< 4	< 4	< 4	
Vitamin D2 and D3 (IU/100 g)	< 4	< 4	< 4	< 4	< 4	
Folic acid (µg/100 g)	< 5	< 5	< 5	< 5	< 5	AOAC 2013.13
Total Folate (vitamin B9) (mg/100 g)	0.14	0.16	0.18	0.14	0.13	NMKL 111:1985
Methyltetrahydrofolate (µg/100 g)	< 5	6.56	< 5	< 5	< 5	AOAC 2013.13
Retinol (vitamin A) (µg/100 g)	< 21	< 21	< 21	< 21	< 21	EN 12823–1 2014

[5] As reported in the published minutes of the 130th meeting of the working group on novel foods (WG NF 2022), the working group (WG) considered that 'for the purpose of the assessment of NFs, intakes that lead to a significant increase of Mn intake as compared to the background diet are considered of concern. The WG also noted that an assessment of the upper level (UL) for Mn is ongoing (EFSA-Q-2021-00371). Based on experts' judgement and criteria set by the Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (2015) for selecting foods/food groups that contribute significantly to total dietary exposure of a contaminant or toxin, the WG concluded that Mn intake above 5% as compared to the high mean background intake (EFSA NDA Panel, 2013) is considered as a significant contribution'.

Parameter (unit)	Batch number (barley-to-rice ratio = 70:30)					Analytical method
	#5	#24	#19	#25	#35	
Thiamine (vitamin B1) (mg/100 g)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	BS EN 14122–2014
Thiamine calculated as thiamine chloride, hydrochloride – (mg/100 g)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	BS EN 14122–2014
Cyanocobalamin (µg/100 g)	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	HPLC (Immuno) Food & Feed Method: J. AOAC 2008, vol 91 No 4
Riboflavin (vitamin B2) (mg/100 g)	0.02	0.03	< 0.01	< 0.01	0.03	EN 14152:2014 mod.
Niacin (vitamin B3) (mg/100 g)	0.197	< 0.1	< 0.1	< 0.1	< 0.1	EN 15652:2009
Pantothenic acid (vitamin B5) (mg/100 g)	0.09	0.16	0.08	0.18	0.10	AOAC 2012.16
Pyridoxine hydrochloride (mg/100 g)	0.159	NR	0.025	0.026	NR	EN 14164:2014
Biotin (vitamin B8) (µg/100 g)	55.1	82.8	72.6	61.1	76.1	Microbiological assay (LST AB 266.1,1995; analog. to FDA method)
Ascorbic acid (vitamin C) (mg/100 g)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	Food Chemistry, 94 (2006) 626–631
alpha-Tocopherol (vitamin E) (mg/100 g)	< 0.08	< 0.08	< 0.08	< 0.08	0.20	EN 12822:2014
Phylloquinone (vitamin K1) (µg/100 g)	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8	EN 14148:2003 mod.

NF: novel food; AOAC: Association of Official Analytical Chemists; NMKL: Nordic-Baltic Committee on Food Analysis; EN: Europaische Norm (European Standard); NR: not reported; FDA: Food and Drug Administration; ICP: Inductively Coupled Plasma; LC–MS/MS: Liquid Chromatography–tandem Mass Spectrometry; HPLC: high-performance liquid chromatography.

Table 14: Minerals and vitamins in the NF with a barley-to-rice ratio – 45:55

Parameter (unit)	Batch number (barley-to-rice = 45:55)					Analytical method
	#12	#13	#14	#10	#11	
Iron (mg/100 g)	1.0	0.8	0.7	1.9	1.2	ICP, AOAC 984.27 mod, 927.02 mod, 985.01 mod, 965.17 mod
Potassium (mg/100 g)	791	869	695	808	757	
Sodium (mg/100 g)	1540	1480	1320	1160	1140	
Calcium (mg/100 g)	146	141	160	28	31	
Magnesium (mg/100 g)	78	84	82	75	72	
Phosphorus (mg/100 g)	110	120	130	110	100	
Copper (mg/100 g)	1.50	0.91	1.60	0.64	0.60	
Manganese (mg/100 g)	0.10	0.10	0.11	0.15	0.12	
Zinc (mg/100 g)	2.70	3.40	3.90	0.53	0.52	
Silicon (mg/100 g)	13	9.9	19	35	34	
Sulphur (mg/100 g)	950	960	1,000	1,000	930	
Vitamin D2 (IU/100 g)	< 4	< 4	< 4	< 4	< 4	LC–MS/MS, Huang et al., Rapid Commun. Mass Spectrum 2014, 28
Vitamin D3 (IU/100 g)	< 4	< 4	< 4	< 4	< 4	
Vitamin D2 and D3 (IU/100 g)	< 4	< 4	< 4	< 4	< 4	
Folic acid (µg/100 g)	6.91	5.65	< 5	< 5	< 5	AOAC 2013.13
Total Folate (vitamin B9) (mg/100 g)	0.09	0.10	0.07	0.13	0.17	NMKL 111:1985

Parameter (unit)	Batch number (barley-to-rice = 45:55)					Analytical method
	#12	#13	#14	#10	#11	
Methyl tetrahydrofolate (μg/100 g)	< 5	< 5	< 5	< 5	< 5	AOAC 2013.13
Retinol (vitamin A) (μg/100 g)	< 21	< 21	< 21	< 21	< 21	EN 12823-1 2014
Thiamin (vitamin B1) (mg/100 g)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	BS EN 14122-2014
Thiamin calculated as thiamine chloride, hydrochloride – (mg/100 g)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	BS EN 14122-2014
Cyanocobalamin (μg/100 g)	0.31	0.41	< 0.25	< 0.25	< 0.25	HPLC (Immuno) Food & Feed Method: J. AOAC 2008, vol 91 No 4
Riboflavin (vitamin B2) (mg/100 g)	0.04	0.05	0.03	0.03	0.03	EN 14152:2014 mod.
Niacin (vitamin B3) (mg/100 g)	0.314	0.181	0.217	0.249	0.221	EN 15652:2009
Pantothenic acid (vitamin B5) (mg/100 g)	0.34	0.40	0.42	0.08	0.10	AOAC 2012.16
Pyridoxine hydrochloride (mg/100 g)	0.143	0.149	0.099	0.062	0.081	EN 14164:2014
Biotin (vitamin B8) (μg/100 g)	66.1	77.6	69.3	70.4	66.2	Microbiological assay (LST AB 266.1, 1995; analog. to FDA method)
Ascorbic acid (vitamin C) (mg/100 g)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	Food Chemistry, 94 (2006) 626–631
alpha-Tocopherol (vitamin E) (mg/100 g)	< 0.08	< 0.08	< 0.08	< 0.08	< 0.08	EN 12822:2014
Phylloquinone (vitamin K1) (μg/100 g)	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8	EN 14148:2003 mod.

NF: novel food; AOAC: Association of Official Analytical Chemists; NMKL: Nordic-Baltic Committee on Food Analysis; EN: Europaische Norm (European Standard); NR: not reported; FDA: Food and Drug Administration; ICP: inductively coupled plasma; LC–MS/MS: liquid chromatography–tandem Mass Spectrometry; HPLC: high-performance liquid chromatography.

The applicant determined the concentrations of phytic acid, ergot alkaloids, patulin, trypsin inhibitors, and lectins in several independently produced batches of the NF (Appendix C). Tannins have been analysed (European pharmacopeia 01/2008:208 14) in one batch and the reported concentration was 0.83%. Phytic acid was less than 0.14% in all the NF batches analysed. The sum of ergot alkaloids was found to be below the LOD of the analytical method implemented (LC–MS/MS, LOD = 3 μg/kg) in 5 of the NF samples tested, whereas, in six other NF batches, the levels ranged from 6.2 up to 109.5 μg/kg. Higher ergot alkaloids levels were observed in batches with a barley-to-rice ratio of 45:55, compared to those of 70:30. Patulin was found to be below the LOD of the analytical method implemented (LC–MS/MS, LOD = 20 μg/kg) in all the 14 NF batches tested. Lectin levels were below 0.05 mg/g. Trypsin inhibitors were present in all the NF batches testes, and the respective results ranged from 461 to 3300 (TIU/g). The reported values in the NF are comparable to the occurrence levels of these compounds in other foodstuffs. The detailed results can be found in Appendix C.

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

3.10. Toxicological information

The Panel notes that no toxicological studies with the NFs were provided. Taking into account the production process, the extensive compositional characterisation of the NF, and the history of use of the source the Panel considers that no toxicological studies are required on the NF.

3.10.1. Human data

No human studies were provided with the NF.

3.11. Allergenicity

Barley *(H. vulgare)* is listed as an allergen subject to labelling according to Annex II of Regulation (EU) No 1169/2011[6].

Allergenic proteins present in barley comprise for example gamma-3 hordein, non-specific lipid transfer protein (nsLTP). Several allergenic proteins are also present in rice (*O. sativa*), such as trypsin alpha-amylase inhibitors, beta-expansin, and profilin A.

Additionally, individuals who are allergic to rice might also show cross-reactivity with other members of the same family, such as barley, oats, wheat, rye, soybean, corn, grass pollen, and triticale (Suvarna, 2008). Correspondingly, barley cross-reactivity has been demonstrated in wheat, rye, oats, and adlay millet (Srisuwatchari et al., 2020).

The applicant did not perform a test to assess the allergenicity of the NF. However, considering the information above, this NF has the potential capacity to sensitise individuals and induce allergic reactions in individuals allergic to barley and/or rice (co-sensitisation or cross-reactivity).

4. Discussion

The NF which is the subject of the application is partially hydrolysed protein from spent barley (*H. vulgare*) and rice (*O. sativa*).

The NF is produced by enzymatically treating the pasteurised spent barley and rice residues of the mash step of beer production. Several mechanical treatment steps (centrifugation, filtering, water evaporation, and spray drying) of the partial hydrolysate are employed to obtain the final product. The NF consists mainly of partially hydrolysed proteins from spent barley and rice. The production process is sufficiently described and does not raise safety concerns. The Panel considers that the NF is sufficiently characterised. The Panel notes that there are no safety concerns regarding the stability as long as the NF complies with the proposed specification limits during its entire shelf life.

The applicant intends to market the NF as an ingredient in several food products. The target population is the general population. Intake 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 children of 3 to < 10 years old, ranging from 229 to 903 mg NF/kg bw per day at the 95th percentile. The Panel notes that consumption of the NF under the proposed uses and use levels does not contribute substantially to the total dietary exposure of the population to the analysed undesirable substances (heavy metals, mycotoxins).

None of the existing ULs for the analysed micronutrients are exceeded considering the proposed uses and use levels. The reported concentrations of antinutritional factors in the NF are comparable to those in other foods.

Considering protein quality aspects, the NF is not a suitable protein source for the nutrition of infants. If the NF entirely replaces other protein sources of higher quality, it might negatively impact protein nutrition also of the other age groups in cases when the overall protein intake is low. Provided that the NF would not be the sole source of dietary protein, that it is integrated into a varied and mixed diet, and considering that the average protein intake in the EU population is high and frequently above DRVs, the consumption of the NF is not expected to negatively impact protein nutrition. 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.

The Panel notes that no toxicological studies or human studies with the NF were provided. Considering the production process, the extensive compositional characterisation of the NF, and the history of use of the source the Panel considers that no toxicological studies are required on the NF.

[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. OJ L 304, 22.11.2011, pp. 18–63.

The Panel considers that the NF has the potential capacity to sensitise individuals and to induce allergic reactions (co-sensitisation or cross-reactivity) in individuals allergic to barley and/or rice. Barley is listed as an allergen subject to labelling according to Annex II of Regulation (EU) No 1169/2011.

5. Conclusions

The Panel concludes that the NF, partially hydrolysed protein from spent barley (*H. vulgare*) and rice (*O. sativa*), 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 following data claimed as proprietary by the applicant:

Report on the protein quality of the NF, information on the inactivation of the enzyme in the NF, information on the absence of mycotoxins and other secondary metabolites produced by *A. niger* in the enzyme preparation, compositional data (certificates of analyses of the NF batches) and the stability study report.

6. Steps taken by EFSA

1) On 10 June 2021, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of proteins obtained from barley (*Hordeum vulgare*) and rice (*Oryza sativa*). Ref. Ares (2021)3810636 – 10/06/2021.
2) On 10 June 2021, a valid application on proteins obtained from barley (*Hordeum vulgare*) and rice (*Oryza sativa*), which was submitted by Evergrain, LLC, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/2195) and the scientific evaluation procedure was initiated.
3) On 15 October 2021, 18 July 2022, and 22 March 2023 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 08 July 2022, 23 February 2023, and 14 April 2023 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 23–25 May 2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of partially hydrolysed protein from spent barley (*Hordeum vulgare*) and rice (*Oryza sativa*) as an NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADME	Absorption, distribution, metabolism and excretion
AOAC	Association of Official Agricultural Chemists
AOCS	American Oil Chemists' Society
aw	water activity
BAM	Bacteriological Analytical Manual
BRC	British Retail Consortium
bw	body weight
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFR	Code of federal regulations
CFU	colony Forming Units
DIAAS	Digestible indispensable amino acid score
DIN	Deutsche Institut für Normung (German Institute for Standardization)
DRVs	dietary reference values
EFSA	European Food Safety Authority
EN	Europaische Norm (European Standard)
est	estimated
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration

FSSC	Food Safety System Certification
GC–MS/MS	gas chromatography tandem mass spectrometry
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HPLC-SEC	size exclusion-high-performance liquid chromatography
HPLC-UV	High performance liquid chromatography-ultraviolet detection
HS-GC–MS	headspace-gas chromatography–mass spectrometry
IAA	indispensable amino acids
ICP-MS	inductively coupled plasma mass spectrometry
IC-UV	ion chromatography coupled to spectrophotometric detection
ISO	International Organization for Standardization
LC	liquid chromatography
LC–MS/MS	liquid chromatography-tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
ML	maximum level
Mn	Manganese
MPN	most probable number
MW	molecular weight
ND	not detected
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
NMKL	Nordic-Baltic Committee on Food Analysis
NR	not reported
nsLTP	non-specific lipid transfer protein
OPA	o-pthaldehyde
P95	95th percentile
PCR	polymerase chain reaction
RH	relative humidity
SCF	Scientific Committee on Food
TAC	total aerobic count
TIU	trypsin inhibitor units
UL	Tolerable Upper Intake Level
UV-RID	ultraviolet refractive index detection
WG	Working group
WHO	World Health Organization

Appendix A – Free Amino Acid Profile of the NF

Barley-to-rice ratio	Batch number											
	70:30					60:40		45:55				
Free amino acids (g/kg)*	#27	#28	#29	#5	#19	#31	#30	#36	#14	#13	#12	#10
Glutamic acid (Free)	< 0.5	< 0.5	1.17	< 0.5	< 0.5	0.99	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Glutamine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Glycine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Histidine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Isoleucine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Leucine (Free)	< 0.5	< 0.5	1.31	< 0.5	< 0.5	0.65	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Lysine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Methionine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Ornithine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Phenylalanine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Proline (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Serine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Threonine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Tyrosine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Valine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Alanine (Free)	< 0.5	< 0.5	0.63	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Arginine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Asparagine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Carnosine	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Tryptophan free	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Urea	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Ethanolamine	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Cystin (free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Aspartic Acid	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Citrulline	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Hydroxyproline (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Phosphoserine (Free)	6.2	6.1	6.4	< 0.5	< 0.5	6.7	6.9	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Phosphoethanolamine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Sarcosine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
alpha-Aminoadipic acid (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
alpha-Amino-n-butyric acid (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Cystathionine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
beta-Alanine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
beta-Aminoisobutyric acid (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Homocysteine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
gamma-Amino-n-butyric acid (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
delta-Hydroxylysine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
1-Methylhistidine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Anserine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
3-Methylhistidine (Free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Taurine (free)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

*: Analytical method: EN ISO 13903:2005 mod.; EN: Europaische Norm (European Standard); ISO: International Organization for Standardization.

Appendix B – Detailed amino acid profile analysis of the NF

Barley-to-rice ratio	70:30	60:40		45:55	
Amino acids (g/100 g NF)	Batch number				
	#16	#22	#21	#14	#23
Alanine[(a)]	4.47	4.69	4.60	5.24	5.04
Arginine[(a)]	4.88	4.71	4.87	5.83	5.60
Aspartic acid[(a)]	8.38	8.39	8.58	9.03	8.73
Cystine[(a)]	1.25	1.41	1.44	1.35	1.38
Glutamic acid[(a)]	21.16	23.34	23.72	20.07	19.24
Glycine[(a)]	4.03	4.18	4.07	4.35	4.17
Histidine[(a),*]	2.10	2.12	2.05	2.18	2.13
Isoleucine[(a),*]	3.84	3.96	4.13	3.80	3.72
Leucine[(a),*]	6.93	7.11	7.36	7.49	7.11
Lysine[(a),*]	3.60	3.58	3.72	3.52	3.40
Methionine[(a),*]	2.04	1.96	2.04	2.14	2.23
Phenylalanine[(a),*]	5.28	5.58	5.70	5.28	5.14
Proline[(a)]	9.12	10.56	11.05	7.40	7.47
Serine[(a)]	3.98	3.98	4.12	4.76	4.54
Threonine[(a),*]	3.37	3.47	3.61	3.84	3.69
Tryptophan[(b),*]	1.44	1.43	1.39	1.37	1.35
Tyrosine[(a)]	3.79	3.80	3.94	4.46	4.28
Valine[(a),*]	5.31	5.62	5.67	5.55	5.45

NF: novel food.
(a): AOAC 982.30 modified, HPLC-UV (High Pressure Liquid Chromatography/Ultraviolet detection).
(b): AOAC 988.15, HPLC-UV (High Pressure Liquid Chromatography/Ultraviolet detection).
*: Indispensable amino acids.

Appendix C – Batch-to-batch analysis of antinutrients in the NF

Parameter (unit)	Batch Number														Analytical method
Barley to rice ratio	70:30							60:40		45:55					
	#5	#7	#19	#24	#25	#29	#37	#30	#31	#10	#11	#12	#13	#14	
Phytic acid (%)	< 0.14	/	< 0.14	< 0.14	< 0.14	< 0.14	/	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	< 0.14	Analytical Biochemistry Vol. 77:536–539 (1977)
Sum of ergot alkaloids (µg/kg)	< 3.0 (LOQ)	/	< 3.0	/	/	< 3.0	6.2	6.8	30.0	/	< 3.0	49.1	50.1	109.5	Internal Method, LC–MS/MS
Patulin (µg/kg)	< 20	/	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	Internal Method, LC–MS/MS
Trypsin inhibitor (TIU/g)	1500	2300	1800	461	503	3300	/	/	/	1600	1600	1600	< 1200	< 1000	AOCS Ba 12–75
Lectin (mg/g)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	/	/	/	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	J Food Sci Biotech Vol 15 No. 1 pp. 95–95 2006 mod

AOCS: American Oil Chemists' Society; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LOQ: limit of quantification; /: not provided.

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

Information provided in this Annex is shown in an Excel file (downloadable at https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2023.8064#support-information-section).