Safety of rapeseed powder from Brassica rapa L. and Brassica napus L. as a Novel food pursuant to Regulation (EU) 2015/2283

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Safety of rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. 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 the safety of rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Rapeseed powder will be produced from the seeds of non-genetically modified double low (00) cultivars that are varieties with a low content of erucic acid and reduced content of glucosinolates compared to older varieties. The applicant developed a production process designed to further reduce the content of glucosinolates and other undesirable compounds such as phytates. The NF will be used as a food ingredient added to a number of food products. The target population is the general population from 1 year of age. The maximum estimated intake of the NF is 18–21 g/day in adolescents, adults and elderly (corresponding to 0.35, 0.23 and 0.25 g/kg body weight (bw) per day, respectively). The levels of undesirable compounds in this NF, such as erucic acid, glucosinolates and phytates, are below levels which would raise concerns. The EFSA NDA Panel has previously assessed the safety of similar products for human consumption and there is extensive experience on the use of rapeseed in animal feed. The applicant provided a human study on the safety and tolerability of the NF and no safety concerns were identified. The Panel considers that the NF, i.e. rapeseed powder from *Brassica rapa* L. and *Brassica napus* L., is safe at the proposed conditions of use.

Keywords: Novel Foods, rapeseed, *Brassica*, safety

Requestor: European Commission

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1. Introduction

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

On 31 December 2018, the company Avena Nordic Grain Oy submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place on the EU market rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. as a novel food. The rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. is intended to be used in a number of food categories.

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 rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. 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 requests for supplementary information.

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[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application.[3] As indicated in this guidance, it is the duty of the applicant to provide all the available (proprietary, confidential and published) scientific data, including both data in favour and not in favour to supporting the safety of the proposed 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 result of a study on the evaluation of safety and tolerability of a rapeseed food ingredient among generally healthy consumers – a randomised double-blind, controlled parallel-group 4-week intervention trial.

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.

An extensive literature search using the following scientific databases: 'Scopus', 'Pubmed', 'Scifinder' and 'Web of Science' was conducted by EFSA following defined search strategies and a standard operating procedure (Dibusz and Vejvodova, 2020). This provided the basis for identifying scientific evidence available in peer-reviewed scientific papers in relation to substances contained in this NF with regard to potential concerns, toxicological data and studies reporting adverse health outcomes in humans.

This assessment concerns only 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.

[1] Regulation (EU) No 2015 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.

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

[3] EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), Turck D, Bresson J-L, Burlingame B, Dean T, Fairweather-Tait S, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Siani A, Sjödin A, Stern M, Tomé D, Vinceti M, Willatts P, Engel K-H, Marchelli R, Pöting A, Poulsen M, Salminen S, Schlatter J, Arcella D, Gelbmann W, de Sesmaisons-Lecarré A, Verhagen H and van Loveren H, 2016. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. https://doi.org/10.2903/j.efsa.2016.4594

3. Assessment

3.1. Introduction

The NF, which is the subject of the application, is the powder produced from the seeds of non-genetically modified (non-GM) double low (00) cultivars of *Brassica rapa* L. and *Brassica napus* L. grown in Europe. The NF is proposed to be used as food ingredient added in 'cereal bars mixed', 'muesli and similar mixed breakfast cereals', 'extruded breakfast cereal products', 'snacks other than chips and similar', 'gluten free breads (brown)', 'bread and rolls with special ingredients added', 'multigrain breads and rolls', 'meat imitates' and 'meat balls'. The intended level to be added is 7–20%. The target population defined by the applicant is the general population (from 1 year of age).

The applicant indicates that, as defined by Regulation (EU) 2015/2283, Article 3 (iv), the NF 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 assessment of the dossier is based on the data presented by the applicant in the dossier for authorisation of the NF in the context of Regulation (EU) 2015/2283. It is also noted that a Scientific Opinion on the safety of 'rapeseed protein isolate' as a Novel Food has been published by the EFSA NDA Panel (2013).

3.2. Identity of the NF

The NF is a powder produced from the seeds of non-genetically modified (GM) double low (00) cultivars of *Brassica rapa* L. and *Brassica napus* L. These species belong to the *Brassicaceae* family. Double low cultivars are varieties with a low content of erucic acid (< 2% expressed as percentage of total fatty acids) and reduced content of glucosinolates (< 25 mmol/kg at a moisture content of 9%) under Regulation (EC) No 2316/1999.[4] The double low cultivars may be used for the production of rapeseed oil for food use (Codex Alimentarius, 1999, 2017).

The common names used are oilseed rape for *B. napus* and turnip rape for *B. rapa*. The name of canola was introduced for double low (00) cultivars in Canada. The name of double low (00) colza may also refer to rapeseed powder from the oilseed rape and the turnip rape.

The applicant states that rape crops for production of the NF are cultivated using authorised seeds of double low (00) cultivars. These varieties are used by European operators manufacturing food grade rapeseed oil.

3.3. Production process

The NF is produced according to Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The quality specification of rapeseed seeds hereafter referred to as 'rapeseeds', the fertilisers and plant protection products that are used for its production are set by EU and national regulations. Rapeseeds are dried after harvest to less than 9.5% moisture content. After heat-drying, the crops are cooled down and stored in ventilated silos that are protected from light and at temperatures below 40°C. Seeds used for NF production are analysed for pesticide residues and heavy metals. The maximum concentration of botanical impurities is 3% and the maximum limit of moisture is 9%. Chlorophyll content should be below 50 ppm, while the portion of free fatty acids should not exceed 2% as oleic acid. Overall, rapeseeds should comply with quality criteria and legal limits set for the placing on the market of food grade oil, press cake and meal for feed materials.

[4] Commission Regulation (EC) No 2316/1999 of 22 October 1999 laying down detailed rules for the application of Council Regulation (EC) No 1251/1999 establishing a support system for producers of certain arable crops.

Rapeseeds including hulls are screw-pressed to obtain partially de-oiled press cake and rapeseed oil. The cake is considered microbiologically stable because moisture content is below 5% and the water activity is between 0.17 and 0.2. Because of this low water activity, endogenous enzymes (e.g. lipase, myrosinase) are not active. Press cake may be stored or transported in sealed cans to other facilities for further processing.

The press cake undergoes an additional water-ethanol extraction step to eliminate glucosinolates. Sugars, starch, lipids and phenolic compounds are also partially extracted by this process. After extraction, the material is prepared, through washing, filtering, centrifugation and acidification (hydrochloric or citric acid), for subsequent enzymatic treatment. A food grade 3-phytase (Maxamyl™) is added at the concentration of 0.04% in dry matter. Following 3 hours of treatment, the enzyme is inactivated by heating at 80°C for 10 min. The Maxamyl™ phytase is produced by a selected *A. niger* strain and is registered as a substance under REACH Regulation (ECHA, 2019). This enzyme is also authorised as a feed additive (e.g. EFSA, 2006; EFSA FEEDAP Panel, 2019) and the FAO/WHO expert group on food additives (JECFA) has evaluated its safety with no indications of safety concern (Choudhuri et al., 2012). Furthermore, this enzyme is authorised for use in baking in France.[5]

As a final step, the material is dried to a moisture content below 6%, usually in an oven, and it is then grounded, as drying causes formation of dry clumps, and packed in bags.

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

3.4. Compositional data

The NF is a brown odourless powder and the major constituents are vegetable oil (18–21%), dietary fibre (37–43%) and protein (34–41%).

The applicant provided analytical information of the NF from seven independent batches (Table 1).

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

Parameters	Units	Batch Number						
		#1	#2	#3	#4	#5	#6	#7
Moisture	%	3.1	3.1	3.7	2.5	2.0	1.4	1.5
Crude protein	%	35.2	34.2	37.4	38.5	41.0	40.1	37.8
Crude fat	%	18.6	20.9	17.4	20.8	19.4	21.1	19.5
Free fatty acid	% as oleic acid	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Ash	%	3.6	3.4	ND	3.5	3.4	3.7	3.3
Carbohydrates*	%	39.5	38.4	ND	34.7	34.1	33.7	37.9
Dietary fibre	%	37.1	42.9	41.0	40.9	38.4	37.4	42.6
Soluble	%	1.1	0.9	2.9	2.6	2.2	2.2	2.4
Insoluble	%	36.0	42.0	38.1	38.3	36.2	38.4	40.2
Peroxide value**	mEkv O_2/kg	0.53	0.72	ND	0.37	0.26	0.46	0.29
Cadmium	mg/kg	0.12	0.14	0.20	0.16	0.17	0.11	0.09
Lead	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Mercury	mg/kg	< 0.005	0.008	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Arsenic	mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Aluminium	mg/kg	27	7.1	33	24	ND	ND	15

ND: not determined.
*: By difference: 100% – [protein % + moisture % + fat % + ash %].
**: Lipid oxidation measurement by method NMKL 158:1997.

The applicant provided detailed analyses for amino acids and proteins, carbohydrates, lipids, vitamins and minerals, erucic acid, phenolic compounds, phytate, glucosinolates, heavy metals, pesticides, dioxins, aflatoxins, polyaromatic hydrocarbons, process contaminants (e.g. ethanol) and for the microbiological quality.

The applicant analysed the amino acid composition of several batches of the NF and compared those with two products containing rapeseed proteins, one that obtained GRAS status in the USA

[5] https://www.legifrance.gouv.fr/affichTexte.do?cidTexte=LEGITEXT000020667468

(GRAS, 2016; and named here RP#1) and one that was previously assessed by EFSA (EFSA NDA Panel 2013; and named here RP2#) that are shown in Table 2.

Table 2: Amino acid composition in RP#1, RP#2 and in five batches of the NF by method ISO13903:25

	Batch number						
	RP#1	**RP#2**	**#1**	**#2**	**#3**	**#4**	**#7**
Total g/100 g	ND	ND	33.4	29.0	36.8	36.6	38.4
Alanine	4.4	4.4	5.1	5.2	5.1	5.0	4.9
Arginine	6.7	6.8	6.7	6.9	6.5	6.5	6.5
Asparagine	6.0	Aspartic acid 7.6	8.5	8.5	8.5	8.5	8.3
Glutamine	23.7	Glutamic acid 22	16.1	16.7	16.8	16.6	17.0
Glycine	5.1	5.1	5.6	5.8	5.7	5.7	5.6
Histidine	3.2	3.5	2.8	2.9	2.7	2.7	2.8
Hydroxyproline	0.0	–	1.1	1.4	1.0	1.0	1.0
Isoleucine	3.7	3.8	4.5	4.6	4.4	4.5	4.4
Leucine	7.3	7.1	8.1	8.2	8.1	8.1	8.0
Lysine	6.4	5.8	6.0	6.6	5.8	5.9	6.1
Phenylalanine	3.9	4.0	4.6	4.7	4.5	4.6	4.5
Proline	7.0	6.8	5.9	6.2	6.2	6.1	6.3
Serine	4.1	5.1	5.0	5.1	5.1	5.0	5.0
Threonine	4.0	4.3	5.3	5.5	5.2	5.2	5.1
Tyrosine	2.1	2.9	3.5	3.6	3.3	3.4	3.3
Valine	5.0	4.8	5.9	6.1	5.8	5.7	5.7
Cysteine+cystine	3.7	Cysteine 2.9	1.7	1.9	1.8	1.7	1.9
Methionine	2.2	2.0	2.1	2.1	2.0	2.1	2.1
Tryptophan	1.4	1.4	1.6	1.8	1.5	1.5	1.5

ND: not described.

Major rapeseed proteins are albumins, globulins and oleosins (Wanasundara et al., 2012). The applicant determined the relative amount of these proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The percentage distribution was approximately 45–35% cruciferin, 25–30% oleosin, 10–30% napin and ~5% late embryogenesis abundant proteins and other proteins. This is comparable with protein compositions of rapeseed products already assessed (EFSA NDA Panel, 2013; GRAS, 2016).

Dietary fibre constitutes the main carbohydrate fraction of the NF. The production process reduces the content of mono- and disaccharides to 0.08–0.14 g/100 g and the content of starch below 0.5 g/100 g. The dietary fibre fraction mainly consists of cellulose, lignin, hemicellulose and other carbohydrate material bound to these. In addition to the data on dietary fibre in Table 1 measured by an enzymatic-gravimetric method, the applicant provided data obtained on acid detergent fibre (25–29 g/100 g), and data on lignin (9.7–13 g/100 g) and uronic acid (6.6–6.9 g/100 g).

Lipid composition was characterised by an analysis of fatty acid composition of triacylglycerols and phospholipids (Table 3). Content of phytosterols varied from 179 to 206 mg/100 g in the NF. Overall, the data were consistent with the composition of food grade low erucic acid rapeseed oil (OECD, 2011).

Table 3: Total lipid and fatty acid composition (fatty acid % of total) in five NF batches by In-house method LAB-M1836, GC-FID

	Batch number				
	#1	#2	#3	#4	#7
Total lipids (g/100 g)*	18.6	20.9	18.1	20.8	19.5
Fatty acid profile %					
Myristic acid (14:0)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Palmitic acid (16:0)	4.3	4.6	4.3	4.4	4.5
Palmitoleic + isomers (16:1 n-7 cis)	0.4	0.3	0.4	0.4	0.4
Stearic acid (18:0)	1.8	1.7	1.7	1.8	1.6
Oleic acid (18:1 cis)	58.6	61.5	57.6	58.6	61.1
Linoleic acid (18:2 n-6 cis)	21.0	19.6	21.9	21.6	20.1
Arachidic acid (20:0)	0.6	0.6	0.6	0.5	0.5
Gondoic acid (cis n-9, 20:1)	1.2	1.1	1.1	0.9	1.1
Alpha linolenic acid (18:3 n-3 cis) (ALA)	10.7	9.4	10.9	10.1	9.5
Behenic acid (22:0)	0.3	0.3	0.3	0.2	0.3
Lignoceric acid (24:0)	0.1	0.1	0.1	0.1	< 0.1
Nervonic acid (24:1 cis)	0.2	0.2	0.2	0.2	0.1
Erucic acid (C22:1n-9)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

*: Total lipids include both major triacylglycerols and phospholipids, and lipid soluble vitamins.

Vitamins (tocopherols and water-soluble vitamins) and minerals (phosphorus, potassium, calcium, magnesium, sodium, iron, sulphur) profiles were considered by the applicant. The tocopherols analysed were alpha-tocopherol (vitamin E) and gamma-tocopherol, and their concentrations were 4.8–5.7 mg/100 g and 2.4–6.5 mg/100 g (method EN12822:2014), respectively. Table 4 reports the content of minerals analysed in five batches of the NF.

Table 4: Content of minerals (mg/kg) in five NF batches by method DIN EN ISO 11885

	Batch number				
	#1	#2	#3	#4	#7
Phosphorus	6,800	6,300	9,000	7,200	6,400
Potassium	2,800	3,900	3,600	3,800	3,200
Calcium	6,400	6,100	7,400	6,100	5,800
Magnesium	2,800	3,000	3,700	3,000	3,200
Sodium	33	24	35	36	35
Iron	130	110	130	160	130
Sulfur	4,500	4,100	4,600	4,900	5,100

The double low (00) rapeseed cultivars are characterised by erucic acid concentrations lower than 2% of total fatty acids. Erucic acid concentration in the NF was below the limit of detection (LOD < 0.1% of total fatty acid) (see Table 3).

Phenolic compounds were also analysed. Sinapine is the major soluble phenolic compound in rapeseed hulls and dehulled flour (Liu et al., 2012). Tannins (proanthocyanidins) provide the dark brown colour in rapeseed hulls (Khajali and Slominski, 2012). The applicant used an HPLC/UV method previously described by Hellström and Mattila (2008) and Mattila et al. (2018). Sinapine content was 0.14–0.19%. Content of free ferulic acid was 86–134 mg/kg and *p*-coumaric acid concentration was below the limit of detection (< 10 mg/kg). Proanthocyanidins content was 214–270 mg/100 g. The applicant carried out an absorption, distribution, metabolism and excretion (ADME), nutrition and toxicological assessment of proanthocyanidins and sinapine compounds (see Sections 3.8, 3.9 and 3.10).

Phytates were analysed after the enzymatic phytase treatment in the production process. The contents were 0.4–1 g/100 g in the NF batches and consistent with those in the specifications of the rapeseed protein previously authorised as an NF (EFSA NDA Panel, 2013).

Glucosinolates are present in *Brassicaceae* vegetables (cauliflower, cabbages, broccoli, horseradish, turnip, kale, Brussels sprouts and mustard seed) and oilseeds (*Brassica napus* and *Brassica rapa*) providing the characteristic flavour and bitter taste of these foods. The production process includes a step to reduce the concentration of glucosinolates in the NF. The residual content (see Table 5) was lower than that identified in the specifications of rapeseed protein NF (EFSA NDA Panel, 2013). Isothiocyanates were not analysed in the NF as they were not considered relevant. This is because isothiocyanates are volatile and water soluble, and therefore, they are expected to largely disappear after the processing steps using high temperatures and soaking in water. Glucosinolates are further considered in the nutritional and toxicological assessment (see Sections 3.9 and 3.10).

Table 5: Concentrations of residual glucosinolates (mmol/kg) in seven batches of the NF by method ISO9167-1:1992; AOCS Ak 1-92

	Ave_pat_2	Ave_pat_3	E130918	E260918	E270918	E280918	E290918
Total	0.09	0.27	< LOQ	< LOQ	0.06	< LOQ	0.26
Progoitrin	0.09	0.19	< 0.05	< 0.05	0.06	< 0.05	0.19
Gluconapin	< 0.05	0.08	< 0.05	< 0.05	< 0.05	< 0.05	0.07

Protease inhibitors were not analysed in the NF. According to the literature, heating at 100°C for 10 min reduced protease inhibitor activity in soybean by about 80% (Gilani et al., 2005). Therefore, protease inhibitors in this NF are also expected to be partially inactivated following the aqueous ethanolic extractions and enzyme inactivation steps in the production process.

Lead, mercury and arsenic content levels in the NF (see Table 1) were below legal limits set for other similar type of foods that are regulated.[6] [,7] Cadmium in one of the batches reached the maximum limit set out for wheat bran for direct consumption according to Commission Regulation (EC) No 1881/2006 and aluminium showed relatively high variability among the batches, so specifications were set for these two compounds. An intake assessment was carried out and compared to other foods in order to contextualise the safety profile of the NF (see Section 3.7.5).

Pesticides, dioxins, aflatoxins and polyaromatic hydrocarbons were analysed and found to be below the applicable legal limits[6,7] for this type of food or for similar foods. Pesticides were not detected in five batches of the NF using the pesticide screening by LC-MS for fatty foods (Method § 64 LFGB L 13.04-5). Dioxins levels can be found in Table 6. Aflatoxins were below detection limits in all the five batches analysed (total aflatoxins < 0.4 μg/kg, by method DIN EN 14123). Finally, the content of polyaromatic hydrocarbons was below the detection limit (< 0.5 μg/kg; by GC-MS). Therefore, these are in compliance with maximum limits for similar foods set out in Regulation (EC) No 1881/2006.

Table 6: Concentration of dioxins and dioxin-like PCBs in five batches of the NF by method GC-MS/MS

	Unit	Batch number					ML*
		#1	#2	#3	#4	#7	
Sum of dioxins (WHO-PCDD F-TEQ)	pg/g fat	0.0682	0.0674	0.068	0.0675	0.0685	0.75
Sum of dioxins and dioxin-like PCBS (WHO-PCDD/F-PCB-TEQ)	pg/g fat	0.0409	0.0405	0.0418	0.0422	0.0411	1.25
Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)	ng/g fat	0.396	0.392	0.395	0.399	0.398	40

*: Maximum Levels according to Regulation 1881/2006 and applicable for vegetable oils and fats.

Other residues from the production process analysed were phytase activity (phytase activity < 180 ftu/kg (LOQ), by method EN ISO30024) and ethanol content (maximum concentration of 14 mg/kg, by, HS-GC-FID). Sulfur dioxide was not detected, and gluten concentrations were within the limits set for food to be labelled as gluten free.

[6] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

[7] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

The NF is proposed to be added to products that will undergo heat treatment at > 130°C. To address the issue of formation of heat-induced process contaminants, the applicant compared acrylamide concentrations in gluten free and leavened breads with and without 7% rapeseed powder before and after toasting. Acrylamide contents were not higher in products manufactured with rapeseed powder. The maximum content for breads with rapeseed powder was observed for toasted leavened bread (25 μg/kg) which is well below the benchmark levels set out for soft bread in Commission Regulation (EU) 2017/2158.[8]

Finally, the applicant analysed the microbiological quality of the NF as a critical control point (Table 7).

Table 7: Microbiological quality of seven batches of the NF

	Units	Batch Number						
		#1	#2	#3	#4	#5	#6	#7
Total plate count 30°C	cfu/g	200	200	200	40	30	30	30
Yeast and mould count	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100
Enterobacteriaceae	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Total coliform count 30°C or 37°C	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Escherichia coli	negative/10 g	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Salmonella	negative/25 g	ND/25 g	ND/25 g	ND/25 g	ND/25 g	ND/25 g	ND/25 g	ND/25 g
Bacillus cereus	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100
Coagulase-positive *Staphylococci*	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100
Clostridium perfringens	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Sulfite-reducing Clostridia	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10

ND: not detected; cfu: colony-forming units.

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

3.4.1. Stability

The applicant performed stability tests with the novel food under accelerated conditions as well as under the conditions of use.

The tests carried out under accelerated conditions were at 40 ± 2°C and at 25–62% of relative humidity (RH) for a period of 5–8 weeks with four independent batches of the NF. The batches were analysed for microbiological quality, peroxides, aldehydes (p-anisidine value) and tocopherols. Microbiological quality was stable under the accelerated conditions, which is explained by the low water content and water activity in the NF. Content of free fatty acids was below the limit of detection. Peroxides were at the limit of the specifications after 5 weeks in the accelerated conditions. The tocopherol profile was analysed to investigate degradation of lipid soluble antioxidants under accelerated conditions, indicating an oxidation of the NF material. Alpha-tocopherol decreased from 5.2–6.1 mg/100 g to 2.8–4.0 mg/100 g after 5 weeks and to 0.29–1.47 mg/100 g after 8 weeks in accelerated conditions.

The accelerated studies showed oxidation of the NF oil and decomposition of tocopherols. However, the applicant reports no sensory changes owing to the oxidation in this study. The intended shelf-life proposed is 6 months at ambient temperature and humidity.

Eight batches of the NF were also stored for 6 months at ambient temperature in the intended package and analysed for its oxidative stability. Four of these batches had been produced by using hydrochloric acid in the acidification step prior to the phytase treatment. The other four batches had been obtained by replacing hydrochloric acid by citric acid. This replacement resulted in enhanced oxidative stability as expressed by the peroxide and the p-anisidine values. Formation of peroxides was within the specification limit after 6 months storage at room temperature (< 3 mEkv O_2/kg by weight) for the batches produced with citric acid packed properly with a minor portion of air, and the

[8] 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

p-anisidine value remained below 5 (measured by spectrophotometry, method AOCS cd 18-90) under these conditions for all the eight batches. Microbiological quality was stable at the ambient conditions of storage.

The applicant also analysed potential degradations under representative proposed conditions of use. These conditions of use can be found in Appendix A. Oxidative stability did not change compared to foods without added NF except for breakfast cereals with 20% added rapeseed powder for which an oxidised odour and an increased peroxide value were observed after 3 months at accelerated conditions (40°C, 60% humidity). The applicant suggested the possible use of authorised antioxidants in this food category, e.g. tocopherols, to increase the oxidative stability in the final food product.

The storing conditions are recommended to be maintained below 30°C and 60% relative humidity. The intended shelf-life time for the NF proposed is 6 months from the date of manufacture.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for 6 months at ambient temperature and humidity.

3.5. Specifications

The NF is a brown and dry powder constituted by particles of a size below 1 mm. The specifications of the NF are indicated in Table 8.

Table 8: Specifications of the NF as proposed by the applicant

Parameter	Limit values	Method
Particle size	< 1 mm	Dry sieving (various methods available)
Moisture	< 7%	NMKL 23:1991; gravimetric determination
Protein (N x 6,25)	33–43% (average 38%)	Kjeldahl, NMKL 6 4 th Ed.:2003
Lipids	14–22% (average 18%)	NMKL 160:1998; Fat. Determination in foods
Ash	2–5%	NMKL 173:2005, 2nd Ed; Gravimetric determination
Water-soluble LMW fibre	< 0.3%	AOAC 2011.25 (McCleary et al., 2011); Enzymatic gravimetry
Water-soluble HMW fibre	0.5–3%	AOAC 2011.25 (McCleary et al., 2011); Enzymatic gravimetry
Water-insoluble HMW fibre	33–41%	AOAC 2011.25 (McCleary et al., 2011); Enzymatic gravimetry
Total fibre	33–43% (average 38%)	AOAC 2011.25 (McCleary et al., 2011); Enzymatic gravimetry
Sugars	< 0.3%	AOAC 982.14 High-performance chromatography
Lignin	< 13%	Ankom Lignin
Acid detergent fibre	< 28%	Ankom ADF 05/03
Gluten	< 20 ppm	r-biopharm Test-Combination R7001:2015-10; Sandwich ELISA
Total glucosinolates	< 0.3 mmol/kg (< 120 mg/kg)	Glucosinolate in Canola Products Method: ISO 9167-1:1992; AOCS Ak 1-92; high-performance liquid chromatography
Phytate	< 1.5%	LC-RI (Graf and Dintzis, 1982)
Proanthocyanidins	< 3,000 mg/kg	(Hellström and Mattila, 2008; Mattila et al., 2018); high-performance liquid chromatography
Lead	< 0.2 mg/kg	DIN EN 15763:2010 (2010-04); ICP-MS
Arsenic (inorganic)	< 0.2 mg/kg	DIN EN 15763:2010 (2010-04); ICP-MS
Cadmium	< 0.2 mg/kg	DIN EN 15763:2010 (2010-04); ICP-MS
Mercury	< 0.1 mg/kg	DIN EN 15763:2010 (2010-04); ICP-MS
Aluminium	< 35 mg/kg	DIN EN ISO 11885 ICP-OES
Peroxide value in NF weight	< 3 mEkv O_2/kg	NMKL 158:1997, mod.

Parameter	Limit values	Method
Free fatty acids in NF weight	< 0.4% as oleic acid (20 mg KOH/g RP)	NMKL 38:2001, mod. and AOAC Vol II 17th Ed, 2000, 41.1.21, mod.
Total plate count 30°C	< 5,000 cfu/g	ISO 4833-1:2013
Salmonella	negative /25 g	VIDAS Easy Salmonella
Enterobacteria count	< 10 cfu/g	NMKL 144:2005
Yeast and mould count	< 100 cfu/g	ISO 21527-2:2008
B. cereus	< 100 cfu/g	Internal method 536, Bacara

AMC: total aerobic microbial count; TYMC: total yeast and mould count; CFU: colony-forming units; LMW: low molecular weight; HMW: high molecular weight.

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

Rapeseed oil is widely consumed around the world. Rapeseed double low (00) varieties are bred to obtain the low erucic acid and glucosinolates content (OECD, 2011). Standards for oils have been previously described (Codex Alimentarius (1999, 2017).

Key constituents having antinutritional and toxic effects have been evaluated previously (OECD, 2011; EFSA NDA Panel, 2013).

3.6.2. History of use of the NF

Rapeseed is mainly used for oil production. By-products, such as rapeseed meal, are used for feed at restricted inclusion rates because of glucosinolates (EFSA, 2008a; OECD, 2011). Inclusion rates vary depending on diets, from 25% of total feed intake in the ruminant diet, to 2.5% for monogastric animals such as pigs and broilers (EFSA, 2008a). The concentration of glucosinolates was limited to 0.5–1 mmol/kg in whole feed for monogastric animals (EFSA, 2008a).

The applicant refers to two rapeseed-based novel food ingredients authorised in the EU, rapeseed protein[9] and rapeseed oil high in unsaponifiable matter.[10]

3.7. Proposed uses and use levels and anticipated intake

According to the applicant, taking into account the content of dietary fibre, protein and oil of the NF, this NF will partially replace current foods that are contributors of dietary fibre intake (e.g. wheat dietary fibre), protein (soya isolates) and oil (other vegetable oils or oils in nuts).

3.7.1. Target population

The target population proposed by the applicant is the general population (from 1 year of age).

As the NF is intended to be used as an ingredient in standard food categories, the NF can be consumed by any group of the population. Therefore, the safety data and the exposure assessment shall cover all population groups (Commission implementing Regulation (EU) 2017/2469,[11] article 5(6).

3.7.2. Proposed uses and use levels

The NF is proposed to be used as an ingredient in several food products. These food products defined using the FoodEx2 hierarchy, and their maximum use levels are reported in Table 9.

[9] Commission Decision 2014/424/EU; Commission Implementing Decision of 1 July 2014 authorising the placing on the market of rapeseed protein as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council.

[10] Commission Decision 2006/722/EC; Commission Decision of 24 October 2006 authorising the placing on the market of rapeseed oil high in unsaponifiable matter as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council.

[11] 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.

Table 9: Food categories and maximum use levels intended by the applicant

FoodEx2 level	FoodEx2 code	Food category	Max. use level (g NF/100 g)
4	A00FA	Cereal bars mixed	20
3	A00EJ	Muesli and similar mixed breakfast cereals	20
5	A0F4Q	Extruded breakfast cereal products	20
3	A06HL	Snack other than chips and similar	15
5	A005T	Gluten free breads, brown	7
4	A005K	Bread and rolls with special ingredients added	7
4	A005L	Multigrain bread and rolls	7
3	A03TE	Meat imitates	10
5	A03XG	Meat balls	10

3.7.3. Anticipated intake of the NF

EFSA performed an assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 9), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intakes of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 10.

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 https://doi.org/10.2903/j.efsa.2020.6197).

Table 10: Intake estimate 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	66.9	0	359.4
Toddlers	1 to < 3	0	183.1	0	489.3
Other children	3 to < 10	1.8	183.6	0	535.2
Adolescents	10 to < 18	1.9	119.3	0	350.0
Adults	18 to < 65	1.4	90.9	0	235.8
Elderly	65 to < 75	0.8	121.2	0	254.5
Very elderly	≥ 75	0.6	124.5	0	196.3

(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 19/05/2020. 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 19/05/2020. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered).

3.7.4. Combined intake from the NF and other sources

Fibres and phytates were considered the most relevant compounds to be considered in this section.

In relation to fibre, the total fibre content of the NF is 33–43% (average value 38%), as described in the specifications. The NF ingredient will partially replace original ingredients that will lead to an increase of fibre content; its nutritional relevance is assessed in Section 3.9.

Phytate was the additional compound that the applicant focused attention on because of its anti-nutritional characteristics. Considering a content of phytate of 1.5% in the NF and the maximum estimated intake of rapeseed powder of 18–21 g/day in adolescents, adults and elderly, the daily intake of phytate would correspond to 270–315 mg. An assessment is provided in Section 3.9.

3.7.5. Estimate of exposure to undesirable substances

The applicant focused the assessment on the following main substances: cadmium, aluminium, glucosinolates, proanthocyanidins and sinapine.

In the case of cadmium, the NF will mainly replace cereals (e.g. wheat grains, wheat bran, rice), soybean, nuts and oilseeds that contain similar amounts of cadmium and are bound to Regulation (EC) No 1881/2006 defining a limit of 0.2 mg/kg on those products. Nevertheless, the applicant elaborated the case of cadmium, as the content of this substance in one batch reached the legal limits (the other six batches were below the legal limits). Considering the high exposure of the NF for 'other children' as 530 mg/kg bw per day and a level of cadmium of 0.2 mg/kg in the NF, it would account to a high exposure to cadmium of 0.75 μg/kg bw per week. The tolerable intake of cadmium is 2.5 μg/kg bw/week (EFSA 2009). A similar scenario was described by the applicant for aluminium where a limit for this substance in the specifications was proposed at 35 mg/kg. Considering the high exposure of rapeseed powder for 'other children' as 530 mg/kg bw per day and a level of aluminium of 35 mg/kg in the NF, it would account to a high exposure to aluminium of 0.13 mg/kg bw per week. EFSA has established a tolerable intake level for aluminium from dietary intake at 1 mg/kg body weight (bw) per week (EFSA, 2008b). The NF will mainly replace cereal flours and flakes which are already important contributors to the dietary aluminium exposure in the general population (EFSA, 2008b), and is thus not expected to change the aluminium exposure to a relevant degree.

In addition, glucosinolates, proanthocyanidins and sinapine have been previously assessed by EFSA in the context of a rapeseed protein application (EFSA NDA Panel, 2013). In relation to glucosinolates, different populations have been studied in the US and Germany and intake levels ranging from 2 to 29 mg/day have been reported (Steinbrecher and Linseisen, 2009; Ma et al., 2018). Considering the maximum levels of glucosinolates as in the specifications in the NF (0.3 mmol/kg or 120 mg/kg), and the maximum estimated intake of the NF of 18-21 g/day in adolescents, adults and elderly, the daily intake of glucosinolates would correspond to 2–2.5 mg/day. The toxicological potential of glucosinolates is further described in the sections on nutrition (3.9) and toxicology (3.10). Similarly, proanthocyanidins and sinapine concentrations in the NF are comparable to those found in other foods and their assessment is further described in Sections 3.9 and 3.10.

3.7.6. Precautions and restrictions of use

No precautions and restriction of use are considered for this NF relating to its safety.

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

The ADME analysis of this NF focused on protein, fibre as well as other constituents such as glucosinolates, proanthocyanidins, sinapine and phytates because they were considered relevant compounds for a focused toxicological and/or nutritional assessment. Information on ADME is based on *in vitro* studies on digestibility performed by the applicant, studies published in the literature and the previous EFSA opinion on rapeseed protein (EFSA NDA Panel, 2013).

The applicant studied the fate of the NF following human ingestion using three models: (i) a harmonised upper intestinal model for determining digestibility of protein and oil; (ii) an ileal *in vitro* model for studying protein digestibility; and (iii) a colon fermentation model for studying the fate of the dietary fibre.

The upper intestinal model consisted of an incubation in oral digesta followed by gastric and duodenal conditions as previously described by Minekus et al. (2014). The enzymes employed were pepsin, pancreatin with bile salts and pancreatic lipase. Protein digestibility of the NF was compared to that of whey and soya protein. The NF proteins were digested by proteolytic enzymes but to a lesser extent (approximately 30% less efficient) than the whey and soya proteins.

In addition, protein digestibility was further studied using an ileal *in vitro* method developed for studying feed protein digestion as a source of amino acids and nitrogen for growth (Boisen and Fernandez, 1995). In this study, the applicant used several batches of the NF, as well as other foods containing the NF as an ingredient. The NF will be consumed following additional processing treatments, e.g. soaking in water and thermal treatment, that may improve the digestibility. In addition, soy protein concentrate and wheat bran were also tested for comparison purposes. In this *in vitro* study, protein digestibility of the NF was shown to be comparable to that of wheat bran.

A colon model (adapted from Nordlund et al., 2012) was used for studying the fermentation process of fibre in the NF. In this case, the control used was wheat bran. The profile of the fatty acids released from fibre in the NF was of similar characteristics as that of the wheat bran. Furthermore, the absorption of insoluble rapeseed fibre was considered negligible.

The applicant also performed a literature search to further document ADME for glucosinolates, proanthocyanidins, sinapine and phytate constituents. Briefly, metabolism of glucosinolates and their

breakdown products has been studied previously (EFSA, 2008a; Verkerk et al., 2009). Current evidence shows that intact glucosinolates are not bioactive by themselves, but they may form breakdown products in the human gut (Narbad and Rossiter, 2018; Tian et al., 2018). Toxicokinetic studies of breakdown products such as isothiocyanates have been carried out in rats and mice (Choi et al., 2014) and an EFSA opinion on isothiocyanates is also available (EFSA ANS Panel, 2010). Glucosinolates and their breakdown products are considered further from nutritional and toxicological points of view in the relevant sections.

Phenolic compounds relevant in rapeseed powder such as long-chain proanthocyanidins are not absorbed in the small intestine and are catabolised in the colon by the microbiota (Monagas et al., 2010). Phenolic metabolites formed in the colon are absorbed and mainly excreted in urine (Monagas et al., 2010). According to the applicant, these phenolic metabolites are similar to other related flavonoids and are considered as innocuous products. In the case of sinapine, the metabolism of cinnamic acid structures has been described and hydroxycinnamic acid esters were shown to be hydrolysed into their respective hydroxycinnamic acid and alcohol; these metabolites were considered as innocuous products (EFSA, 2005).

Phytate degradation in the gastrointestinal tract is limited (Schlemmer et al., 2009). It is assumed that in humans, the main phytate hydrolysis occurs in the large intestine by microbial phytases. Phytate also binds to minerals and trace elements in the gastrointestinal tract and forms complexes that are excreted.

3.9. Nutritional information

Major components of the NF are fibre, protein and fat. According to its anticipated use as a food ingredient, it may partially replace other sources of protein, dietary fibre and fat in foods (Section 3.7). The applicant has calculated the change in nutritional profile for different reference foods and rapeseed powder foods including the proposed food categories. As a result, the mean (+/− SD) changes in content of nutrients were + 2 (± 2.8) % for protein, +3 (± 2) % for dietary fibre, +0.2 (± 3.3) % for fat, −6 (± 4.7) % for digestible carbohydrates and −1 (± 7.3) % for energy.

Although the rapeseed amino acid profile of the NF is comparable to authorised rapeseed-containing products (see Table 2; EFSA NDA Panel, 2013), proteins contained in the NF were not digested as efficiently as control proteins in the *in vitro* upper intestinal model (Section 3.8), which was ascribed by the applicant to encapsulation/denaturation of the rapeseed protein and its binding to acid detergent fibre (ADF). Protein digestibility as assessed using an ileal *in vitro* method was found to be comparable to that of wheat bran. The non-digested rapeseed protein will undergo partial fermentation in the colon leading to the formation of small amounts of branched chain fatty acids among other metabolites. Furthermore, the applicant argues that because rapeseed powder will be used as an ingredient in processed foods, the bioavailability of protein will be further improved due to the effect of mixing with other ingredients and the production processes; however, to which extent this effect will take place is not known.

Based on the high (95th percentile) intake levels of rapeseed powder (Section 3.7.3) with a maximum content of protein of 43%, corresponding intakes of rapeseed protein per kg body weight amount to 0.21 g for toddlers, 0.23 g for other children, 0.15 g for adolescents and 0.10 g for adults. These intakes correspond to 18.5–23.4%, 26.8%, 15.5–18.1% and 12% of respective dietary reference values (DRVs) for protein for toddlers, other children, adolescents and adults.

The Panel notes the limited bioavailability of the protein in this NF. If the NF ingredient entirely replaces other protein sources of higher quality and bioavailability, it may negatively impact protein nutrition. However, considering that the average protein intake in EU population is high and frequently above DRVs, this situation is unlikely to occur (EFSA NDA Panel, 2012).

Carbohydrate fibre fermentations in the *in vitro* colon model (Section 3.8) were similar between rapeseed powder and wheat bran. Based on an over-conservative intake scenario (i.e. that combines the high (95th percentile) fibre intake from background diet (EFSA NDA Panel, 2010) of which 30% is replaced and the high (95th percentile) fibre intake from the rapeseed powder foods with the maximum content of fibre of rapeseed powder (43%)), total fibre intake would be 49 g/day in adults, 19 g/day for toddlers, 35 g/d for other children and 47 g/day for adolescents. These intake levels would exceed the DRV for fibre intake (DRVs as adequate intakes (AIs) for fibre amount to 25 g for adults and 10–21 g for children aged 1–17 years (EFSA NDA Panel, 2010)); however, these were based on bowel function, but evidence in adults of benefit to health was noted with consumption of dietary fibre higher than 25 g/day. No studies are available with regard to tolerability of such high

amounts of the NF or other fibres. Apart from gastrointestinal symptoms, which in general may be related to high intake of fibre, no other adverse effects are known. Occurrence of gastrointestinal symptoms or experience of gastrointestinal discomfort in relation to fibre intake differs among individuals and may limit its intakes.

With regard to the fat of this NF, the fatty acid composition mainly consists of oleic acid (58%), linoleic acid (21%) and alpha linolenic acid (10%) which is typical for the food grade rapeseed oil (Codex Alimentarius (1999, 2017).

The Panel notes that the difference in fat intake with foods containing the NF ingredient is minor and that the fatty acid composition is comparable to that of rapeseed oil.

With regard to anti-nutritional factors, the contents of polyphenols such as sinapine and proanthocyanidins in the NF are comparable to those found in other foods (e.g. some nuts, berries, chocolate). Specification of NF for phytates is < 1.5% and thus similar to concentrations in other foods of plant origin, e.g. wheat bran, oat, various beans, chickpeas, soybeans and different types of nuts (Schlemmer et al., 2009). The level of glucosinolates and their breakdown products has been reduced in this NF during the production process to the level of < 0.3 mmol/kg, which is lower than that in the authorised novel food rapeseed protein (< 1 mmol/kg) (Commission Implementing Regulation (EU) 2017/2470). Protease inhibitors were not analysed in this NF. Based on information from the literature, the applicant argues that protease inhibitors are partially inactivated upon heat treatments (Gilani et al., 2005). The relevance of protease inhibitors in rapeseed is of lower importance as compared to other crops. This is further supported by the fact that OECD consensus document on compositional considerations for new varieties of low erucic acid rapeseed (OECD, 2011) does not consider any protease inhibitor because of lack of relevance in this crop. In this context, protein digestibility was also considered in Section 3.8.

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

3.10. Toxicological information

The chemical composition of the NF is mainly composed of proteins, fat and fibre (please see section 3.4). The toxicological assessment was performed following a tiered approach.

Considering the studies on ADME (see Section 3.8), most parts of the NF will be absorbed in the gastrointestinal tract (protein, fat and minor constituents). However, attention was paid to the dietary fibre fraction as it was observed to pass the small intestine and be fermented in the large intestine. This aspect was further assessed in an *in vitro* model (see Section 3.8) and in a human study (see Section 3.10.3) by the applicant. For the overall assessment, additional information was considered using the safety data available on rapeseed protein (EFSA NDA Panel, 2013) and the history of consumption of rapeseed oil. Taking into consideration these elements, specific toxicity studies were not considered necessary for this NF.

In relation to undesirable compounds contained in the NF, it is noted that the NF is rapeseed powder produced from the seeds of non-genetically modified (GM) double low (00) cultivars of *Brassica rapa* L. and *Brassica napus* L. Double low cultivars are varieties of low content of erucic acid (fatty acid basis content < 2%) and of reduced content of glucosinolates (< 25 mmol/kg at a moisture content of 9%).

With regard to erucic acid, considering the NF origin and the batch test results (erucic acid below detection limit of 0.1% of total fat), the Panel considers that the concentration of erucic acid in the NF does not raise concerns.

In relation to glucosinolates, the total concentration of these compounds in the NF is below 0.3 mmol/kg. Assuming a high daily intake scenario of e.g. 0.25 g NF/kg bw for adults and 0.50 g NF/kg bw for toddlers and other children, it would result in maximum intake levels previously considered of no safety concern by EFSA (EFSA NDA Panel, 2013). An adult high consumer of the NF will be exposed to glucosinolates intake lower than the average intake from *Brassica* vegetables in German and Spanish populations (Agudo et al., 2008; Steinbrecher and Linseisen, 2009). Individual glucosinolates were also reviewed and the applicant considered progoitrin as the one with most negative health effects, while isothiocyanates were considered less harmful. This is because glucosinolates degrade to goitrin and thiocyanate which may decrease thyroid hormone production. While increased iodine intake can prevent the adverse effects of isothiocyanates, this is not the case for goitrin (Felker et al., 2016). The total intake of progoitrin from the NF would be lower than those reached with other foods in the overall diet (EFSA NDA Panel, 2013; Felker et al., 2016).

In addition, the applicant also assessed potential adverse effects of proanthocyanidins, sinapine and phytate. The anti-nutritional effect has been described in the previous sections and this NF was considered to be comparable with other foods (Section 3.9). Toxicological effects are comparable to other foods and have been previously assessed by EFSA (EFSA NDA Panel, 2013). Similarly, cadmium and aluminium contents of the NF do not raise safety concerns as the NF will mainly replace cereals, soybean, nuts and oilseeds, containing similar amounts of these compounds (see Section 3.7.5).

3.10.1. Genotoxicity

The applicant provided a genotoxicity study on the NF, which was conducted in compliance with OECD principles of good laboratory practice (GLP) and in accordance with the test guideline No 471 from the Organisation for Economic Co-operation and Development (OECD, 1997).

The applicant provided a bacteria reverse mutation assay with *Salmonella Typhimurium* TA97, TA98, TA100 and TA1535, and *Escherichia coli* WP2 trp UvrA with the batches Ave_pat_2, E130918 and E290928 (Biosafe, 2018). Representative samples were extracted with ethanol at room temperature for 24 hours and concentrated by evaporator and further with stream of nitrogen to achieve 50× concentrated sample. The tests were performed as plate incorporation assays with and without metabolic activation where bacteria were exposed to concentrations of 10 μg/plate, 50 μg/plate, 100 μg/plate, 500 μg/plate, 1,000 μg/plate and 5,000 μg/plate. There was no indication of induction of point mutations or antibacterial effects in the Ames test with or without metabolic activation (S9) at the doses applied. All test strains responded to positive and negative control substances as expected. The applicant argues that rapeseed powder has no genotoxic potential and that Tier 2 *in vivo* genotoxicity tests are not required.

Taking into account the test results provided and considering the nature, source and production process of the NF, the Panel considers that there are no concerns regarding mutagenicity.

An *in vitro* micronucleus test, although recommended in the EFSA Scientific Opinion on genotoxicity testing strategies (EFSA Scientific Committee, 2011), was not provided.

The NDA Panel concludes that, considering the results from the mutagenicity tests submitted, and considering the nature, source and production process of the NF, an *in vitro* micronucleus test can be omitted. The Panel concludes that there are no concerns regarding genotoxicity of the NF.

3.10.2. Subchronic toxicity

No subchronic toxicity study was provided.

3.10.3. Human data

The applicant provided a 4-week randomised, double-blind, controlled, parallel-group intervention of rapeseed powder succeeded by a 2-week follow-up period in 54 healthy subjects (Medfiles, 2018). The objective of the study was to assess the safety and tolerability of the NF with special focus on its fibre content. Thus, the primary objective of this non-inferiority trial was to compare the frequency of gut-associated symptoms among study subjects consuming daily two test bars (2 × 40 g) containing a total of 20 g of NF (consisting of 33–43% fibre) compared with that of study subjects consuming two control bars (containing 9.1% fibre). Background diets were not controlled.

Gut-associated symptoms (abdominal pain/discomfort, bloating, flatulence/passage of gas and borborygmic/rumbling stomach) were assessed at randomisation and thereafter bi-weekly during the intervention and follow-up periods using the Likert scale-based digestive symptom frequency questionnaire (DSFQ), which was considered by the applicant as an appropriate tool because it was validated among generally healthy subjects (Guyonnet et al., 2009, 2013; Azpiroz et al., 2015). All four symptom frequencies were scored 0–4 resulting in a composite score of 0–16. The number of reported adverse events (AEs) according to adapted ICD10 main classes (total and according to causality assessment category), clinically significant changes in blood safety tests, BMI, defecation frequency, average stool consistency and palatability of the test product were evaluated as secondary outcomes.

The non-inferiority margin was *a priori* set to 3 in the DSFQ scores. The power was set at 80% (alpha level at 0.05) and it was calculated that 24 individuals were needed per group (27 per group to be recruited, considering 10% attrition).

For the modified intention-to-treat (ITT) population (N = 53, primary dataset, 26 and 27 in the test and control group respectively), the non-inferiority was shown with both analysis of variance (ANOVA) and analysis of covariance (ANCOVA) with baseline DSFQ score as a covariate. The estimated

difference, between the test and control groups, in change of DSFQ score from baseline, measured at week 4, was 0.542 (95% confidence limits −1.453 and 2.538) with ANCOVA and 0.238 (95% confidence limits −1.791 and 2.267) with ANOVA.

For the per protocol (PP) population (N = 43, 19 and 24 in the test and control group, respectively), the non-inferiority was not shown, neither with ANCOVA (the estimated difference, between test and control groups, in change of DSFQ score from baseline, measured at week 4, was 1.798 with 95% confidence limits at 0.240 and 3.356) nor with ANOVA (the estimated difference between test and control groups, in change of DSFQ score from baseline, measured at week 4 was 1.559 with 95% confidence limits at 0.054 and 3.172). Among the 10 participants excluded from the PP analysis were 6 subjects who did not comply with the consumption of products (lowest use level reported 60%), and 4 subjects who dropped out of the study during the intervention period.

There were no clinically relevant findings within the clinical chemistry or haematological parameters related to safety. The number of AEs and their causality and severity were similar between the two groups. The palatability of the product containing the NF was slightly less favourable, presumably due to the high portion of rapeseed powder in the test bar which made the consistency of the bar drier in comparison to the control bar.

The Panel notes that, for the ITT population, the increase in gastrointestinal symptoms among participants of the test group was not significantly worse than that of the control group and the upper bound of the 95% CI was below the prespecified margin of non-inferiority. This was not confirmed in the PP analysis in which a statistically significant increase in symptoms in the test compared with the control group was observed and the upper bound of the 95% CI exceeded the non-inferiority margin. This did not allow to confirm the non-inferiority hypothesis for this population. The Panel, however, notes that this increase in gastrointestinal symptoms may be explained by the higher fibre content of the test product (14.2 g/daily dose) compared with the control product (7.3 g/daily dose). Gastrointestinal symptoms have been associated with the consumption of other fibre-rich foods and are not specific to the NF. In addition, as the number and severity of AEs other than gastrointestinal symptoms were similar between groups, the Panel considers that the available human data do not point to safety concerns in relation to the NF.

3.11. Allergenicity

Food allergy to rapeseed (*Brassica rapa* L.) and oilseed rape (*Brassica napus* L.) has been reported to occur (Poikonen et al., 2006, 2008; Puumalainen et al., 2006, 2015). Indications of cross-reactivity between rapeseed proteins and other food proteins, particularly those of mustard have been described (Monsalve et al., 1997; Poikonen et al., 2009). An EFSA opinion on the allergenicity of rapeseed protein isolates has already been published and the NDA Panel considered that the risk of sensitisation to rapeseed cannot be excluded and that it is likely that rapeseed can trigger allergic reactions in mustard allergic subjects (EFSA NDA Panel, 2013).

The applicant did not carry out any specific study to determine the potential allergenicity of this NF. Nevertheless, the applicant argues that because the protein fraction of this NF is similar to that of authorised rapeseed protein in the Union list of novel foods (Commission Implementing Regulation 2017/2470), the following statement is proposed by the applicant to be included on the package labels of this NF: *this ingredient may cause allergic reaction to consumers who are allergic to mustard and products thereof*.

4. Discussion

The NF, which is the subject of the application, is rapeseed powder as a food ingredient produced from the seeds of non-genetically modified (non-GM) double low (00) cultivars of *Brassica rapa* L. and *Brassica napus* L. grown in Europe. The cultivars are varieties with a low content of erucic acid (< 2% expressed as percentage of total fatty acids) and a reduced content of glucosinolates (< 25 mmol/kg at a moisture content of 9%). The target population defined by the applicant is the general population (from 1 year of age).

The information provided on the production process, composition and specifications of the NF does not raise safety concerns. The maximum estimated intake of the NF is 18–21 g/day in adolescents, adults and elderly (corresponding to 0.35, 0.23 and 0.25 g/kg bw per day, respectively). According to data provided by the applicant, the Panel notes that the amounts of undesirable compounds in this NF, such as erucic acid, glucosinolates and phytates, are either below detection limits or below levels

which would raise concerns. The applicant included additional steps in the production process designed to further reduce the content of phytates and glucosinolates. The NDA Panel has previously assessed the safety of rapeseed protein for human consumption and no safety concerns were identified under the conditions of use (EFSA NDA Panel, 2013). The NDA Panel considers that it is likely that the NF (rapeseed powder) may trigger allergic reactions in mustard allergic subjects. In addition, there is extensive experience on the use of rapeseed in animal feed. The applicant also provided a human study on the safety and tolerability of the NF and no safety concerns were identified.

The NF which is the subject of the application is considered to be safe under the proposed conditions of use.

5. Conclusions

The Panel concludes that the NF, rapeseed powder, is safe under the proposed conditions of use.

The Panel could 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 ('A randomised double-blind, controlled parallel-group 4-week intervention trial to assess the safety and tolerability of a rapeseed food ingredient among generally healthy consumers').

6. Steps taken by EFSA

1) Letter from the European Commission to the European Food Safety Authority with the request for a scientific opinion on the safety of rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. as a Novel food pursuant to Regulation (EU) 2015/2283, Ref. Ares(2019) 3900330, dated 19 June 2020.
2) On 19 June 2020, a valid application on the safety of rapeseed powder from *Brassica rapa* L. and *Brassica napus* L., which was submitted by Avena Nordic Grain Oy, was made available to EFSA by the European Commission through the Commission e-submission portal (NF2018/0768) and the scientific evaluation procedure was initiated.
3) On 20 December 2019 and 13 March 2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 26 February 2020 and 3 June 2020, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 30 June 2020, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. as a Novel food pursuant to Regulation (EU) 2015/2283.

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Abbreviations

ADF	acid detergent fibre
ADME	absorption, distribution, metabolism and excretion
AEs	Adverse events
AIs	Adequate intakes
AMC	Total aerobic microbial count
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ANS	EFSA Panel on Food Additives and Nutrient Sources added to food
BMI	Body mass index
bw	body weight
CFU	Colony-forming units
DSFQ	Digestive symptom frequency questionnaire
DRVs	Dietary reference values
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GC-MS	Gas chromatography-mass spectrometry
GM	Genetically modified
GLP	Good laboratory practice
GMP	Good manufacturing practice
GRAS	Generally recognised as safe
HACCP	Hazard analysis critical control points
HMW	High molecular weight
HPLC-UV	High-performance liquid chromatography-ultraviolet
HS-GC-FID	Headspace gas chromatography with flame ionisation
ICD10	International Classification of Diseases 10th Revision
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectrometry
ITT	Intention to treat
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS	Liquid chromatography-mass spectrometry
LC-RI	Liquid chromatography-refractive Index
LMW	Low molecular weight
LOD	Limit of detection
LOQ	Limit of quantification
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	Novel food
OECD	Organization for Economic Cooperation and Development
PCBs	Polychlorinated biphenyls
PP	Per protocol
RH	Relative humidity
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TYMC	Total yeast and mould count
WHO	World Health Organization

Appendix A – Processing steps used for manufacturing representative products with the NF as described by the applicant

Food category	Maximum level (%)	Representative constituents, time–temperature and preservatives added
Cereal bars	20	**Constituents:** Glucose syrup, whole grain oat flakes, Rapeseed powder, sunflower seeds, Rapeseed oil, apple pure, cashew nuts, psyllium **Time–temperature:** Liquid constituents (glucose syrup, rapeseed oil, apple pure) were heated until temperature 105 °C was reached. Premixed dry constituents were added and the mass was mixed 2 min, resulting temperature of 63–68°C. Bars were cooled in the precooling chamber 20 min at -18 °C, resulting temperature of 17–18°C.
Muesli and similar mixed breakfast cereals	20	**Constituents:** Whole grain oats, Rapeseed powder, honey, whole grain oat flour, rapeseed oil, coconut oil **Time–temperature:** In the mixing process fats (coconut oil, rapeseed oil) were heated until coconut oil melting point was reached (54°C). Honey and premixed dry constituents (oats, Rapeseed powder) were added to the fat mixture. Mass was dehydrated in the oven (100% dry heat) at 110°C, 390 min and at 130°C, 15 min. Temperature of the product after dehydration was 100–103°C.
Processed and mixed breakfast cereals	20	**Constituents:** Rice flour, Rapeseed powder, caster sugar, salt (NaCl) **Time–temperature**: Premixed flours were extruded in a pilot scale single-screw extrusion machine by using dry extrusion method. Temperature profile and pressure set in the extrusion machine was 33-84-93-110°C and 60–67 bars (6.0–6.7 Mpa). Total cooking time was 30–60 s. After extrusion cereals were dehydrated in the oven (100% dry heat) 100°C, 15 min.
Fried or extruded cereal, seed or root-based products (snacks)	15	**Constituents:** Rice flour, oat bran, Rapeseed powder, caster sugar, salt (NaCl) **Time–temperature:** Premixed dry constituents were extruded in a pilot scale single-screw extrusion machine by using dry extrusion method. Temperature profile set in the extrusion machine was 33-84-93-110°C and cooking time 30–60 s. After extrusion snacks were dehydrated in the oven (100% dry heat) 100°C, 15 min.
Bread alternative	7	**Constituents:** Water, buckwheat flour, Rapeseed powder, buckwheat flakes, potato mash powder, caster sugar, yeast (*Saccharomyces cerevisiae*), salt (NaCl), psyllium **Time–temperature:** Water (40°C) was added to premixed dry constituents. The dough was mixed 5 min with high speed planetary mixer. Dough was dosed to a mould and was let to ferment and proof at proving cabinet at 42°C, 60%, 30 min. Dough was baked at 170°C, 30 min in the oven (100% dry heat).
Leavened bread	7	**Constituents:** Whole grain rye flour, water, Rapeseed powder, yeast (*Saccharomyces cerevisiae)*, salt, starter **Time–temperature:** Liquid sourdoughs were prepared by mixing rye flour, water and starter. Sourdough was fermented for 18 hours at 30°C, resulting pH of 3.6 and total titratable acid (TTA) 17.4 ml. Dough constituents (rye flour, Rapeseed powder, salt, yeast) were mixed with spiral mixer at 2 min (slow) and 2 min (fast), dough temperature 26°C. Dough was proofed in a proving cabinet at 45 min, 28°C, 70 RH% before dividing and 75 min, 35°C, 70 RH% after dividing. Dough was baked 40 min at 230°C (100% dry heat) with 20 s of steam. TTA and pH of the rye bread was 12.7 and 4.5, respectively.
Meat imitates	10	**Constituents:** Water, vital wheat protein, Rapeseed powder **Time–temperature:** Flour mix was extruded in a pilot scale two-screw extrusion machine by using wet extrusion method. Temperature profile set in the extrusion machine was 145-180-145-110-75-65-55°C and cooking time 5–8 min, reaching temperature of 143–145°C.

Food category	Maximum level (%)	Representative constituents, time–temperature and preservatives added
Meat balls	10	**Constituents:** Pork, beef, water, Rapeseed powder, wheat bread crumbs, salt (NaCl), black pepper **Time–temperature:** Premixed dry ingredients were added to ground meat. The mass was mixed 2 min at low speed and 2 min with medium speed in planetary mixer with blade. The mass was shaped to ball shape. Meatballs were cooked in the oven (100% dry heat) 15 min at 170°C, resulting temperature of 80–81°C. Meatballs were cooled in the precooling chamber 30 min at −18°C, resulting temperature of 5°C.