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SCIENTIFIC OPINION

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Safety of rhamnogalacturonan-I enriched carrot fibre (cRG-I) as a novel food pursuant to Regulation (EU) 2015/2283

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

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 rhamnogalacturonan-I enriched carrot fibre (cRG-I) as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is a high molecular weight polysaccharide derived from carrot pomace. The Panel considers that the production process is sufficiently described and does not raise safety concerns. The novel food is intended for use as ingredient in various food products targeting the general population, in food for special medical purposes, meal replacement for weight control and food supplements targeting the general population excluding infants, and in total diet replacement for weight control targeting the adult population. Taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. Based on the data provided the Panel considers that there are no concerns regarding genotoxicity. Results from the 90-day study did not show effects of toxicological relevance for humans up to the highest dose tested (7753 mg/kg bw per day). The NF may retain the allergenic potential of carrots and allergic reactions to the NF may occur, but they will not be dissimilar from those triggered by the consumption of carrots. The Panel considers the margins of exposure to be sufficient considering that the source (i.e. carrots), nature, composition and production of the NF do not raise safety concerns. The Panel concludes that the NF, cRG-I, a rhamnogalacturonan-rich polysaccharide fraction derived from carrot pomace, is safe under the proposed conditions of use.

KEYWORDS

carrot, cRG-I, food for special medical purposes, food supplement, novel foods, pectin, safety, total daily diet replacement for weight reduction

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1 | INTRODUCTION

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

On 17 December 2022, the company NutriLeads B.V. submitted an application to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283 to authorise the placing on the Union market of rhamnogalacturonan-I enriched carrot fibre (cRG-I) as a novel food. The applicant requests to authorise use of rhamnogalacturonan-I enriched carrot fibre (cRG-I) as a novel food in several foods. The applicant has also requested data protection under Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 29(l)(a) of Regulation (EC) No 178/2002, the European Commission asks EFSA to provide a scientific opinion on rhamnogalacturonan-I enriched carrot fibre (cRG-I) as a novel food in accordance with Article 10(3) of Regulation (EU) 2015/2283. The Commission also asks EFSA 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 rhamnogalacturonan-I enriched carrot fibre (cRG-I) as a novel food regarding the proprietary data for which the applicant is requesting data protection.

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.[1]

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2021). 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.

The applicant has submitted a confidential and a non-confidential version of a dossier following the 'EFSA guidelines on the preparation and presentation of a NF' (EFSA NDA Panel, 2021) and the 'Administrative guidance for the preparation of applications on novel foods pursuant to Article 10 of Regulation (EU) 2015/2283' (EFSA, 2021).

In accordance with Article 38 of Regulation (EC) No 178/2002[2] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[3] the non-confidential version of the dossier has been published on Open.EFSA.[4]

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[2] EFSA carried out a public consultation on the non-confidential version of the technical dossier from 1 August to 22 August 2024 for which no comments were received.

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 are listed in Appendix C.

2.2 | Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2021) 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.

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

[2]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

[3]Decision available at: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

[4]The non-confidential version of the dossier has been published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/questions/EFSA-Q-2023-00025.

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.

In the context of this opinion, EFSA's definition of dietary fibre (i.e. non-digestible carbohydrates plus lignin; EFSA NDA Panel, 2010) does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence laid down in Annex I of Regulation (EC) 1169/2011[5] for:

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

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

The safety of the food enzymes used in the manufacture of the novel food is subject to the provisions of Regulation (EC) No 1332/2008, and therefore, it is outside the scope of this opinion, which concerns the assessment of the safety of the novel food according to the provisions of Regulation (EU) 2015/2283.[6] Therefore, the conclusions of the Panel are related to the safety of the specific rhamnogalacturonan-I for which the dossier has been submitted. Conclusions are not related to the safety of the individual enzymes or their combination.

3 | ASSESSMENT

3.1 | Introduction

The NF, in accordance with article 3 of the NF Regulation (EU) 2015/2283, falls under the category (iv) 'food consisting of, isolated from or produced from plants and their parts'. The NF is produced by enzymatic hydrolysis of carrot pomace, followed by the isolation of the branched rhamnogalacturonan-rich structures and consists mainly of soluble dietary fibre.

The novel food is intended for use as ingredient in various food products targeting the general population, in food for special medical purposes, meal replacement for weight control and food supplements targeting the general population excluding infants, and in total diet replacement for weight control targeting the adult population.

3.2 | Identity of the NF

The NF is a high molecular weight polysaccharide derived from the polysaccharide fraction of carrot pomace. The carrot pomace is a by-product of carrot juice production. The species utilised is the commonly consumed carrot (*Daucus carota* L., subspecies *Sativus*), which is included in Directive 2002/55/EC and can be marketed in the EU. The Contract Manufacturing Organization (CMO) guarantees the identity of the carrots.

The NF consists of the rhamnogalacturonan-I rich pectin fraction in the form of water-soluble powder, denominated cRG-I (carrot RG-I). RG-I is naturally present in plant cell walls. The applicant intends to commercialise the product under the name BeniCaros.®

Pectin molecules are high molecular weight polysaccharides, consisting of a backbone of two covalently linked repeating structural units: the linear homogalacturonan (HG) and two types of branched rhamnogalacturonans (RG), RG-I and RG-II. Both branches of RG-I and RG-II are present in carrot pectin. HG consists of α-1,4-linked D-galacturonic acid (GalA) monomers, whereas the backbone of RG-I contains repeating units of the disaccharide [α-1,4-D-galacturonic acid-α-1,2-D-rhamnose-]. Depending on the plant species, the rhamnose residues in the RG-I backbone are substituted with β-1,4-D-galactan, branched arabinan (α-1,5-linked L-arabinofuranose (L-Araf)) units with additional L-Araf sidechains or arabinogalactans (Coenen et al., 2007). A general, schematic overview of a pectin structure with a focus on the localisation of the RG-I motif is given in Figure 1.

[5]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 Text with EEA relevance.

[6]Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes and amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97.

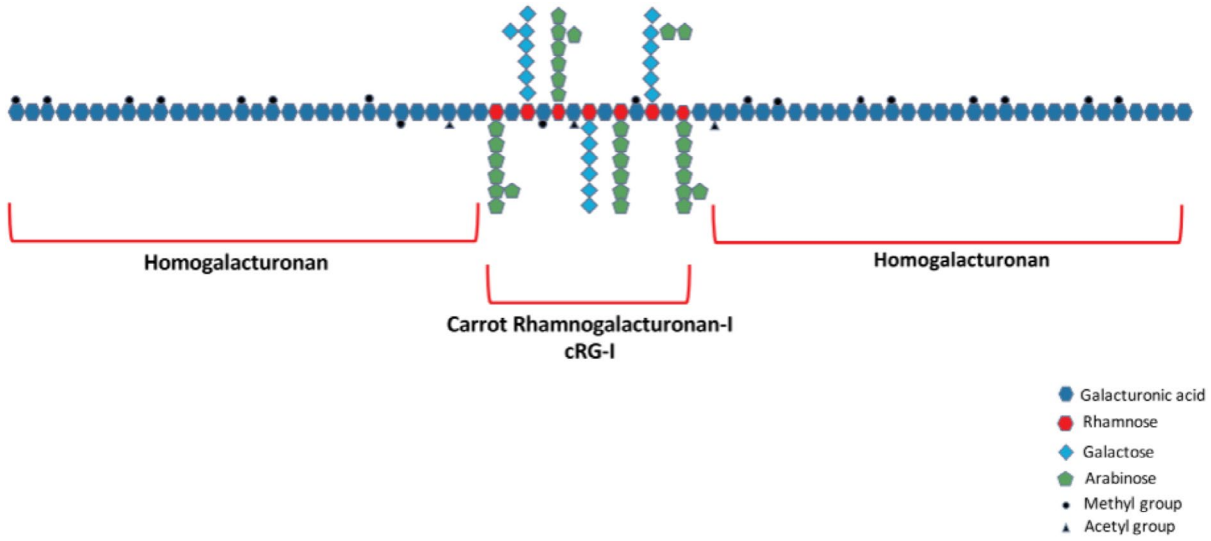


FIGURE 1 Schematic overview of the structure of pectin and the localisation of cRG-I structure (From Jonker et al., 2020).

The NF is a hydrolysate of the pectin fraction that is produced by enzymatic hydrolysis of carrot pomace with a mix of enzymes in order to partially hydrolyse the linear HG structures, while retaining the branched polymeric RG-I. The cRG-I polysaccharides are concentrated by removing sugar monomers and small oligosaccharides by filtration.

The identity of the NF was characterised based on the molecular weight distribution pattern (Appendix A), and the monomer composition (Table 1).

The molecular weight distribution pattern was analysed in 10 batches of the NF by high performance size exclusion chromatography with refractive index detection (HPSEC-RI). Validation of the analytical method and certificates of analyses were provided. Chromatograms showed that the NF is composed of poly- and oligomers with molecular weights between 2 and 1000 kDa.

To further characterise the identity of the NF, the applicant provided monosaccharide composition of cRG-I in 10 batches of the NF, identifying the characteristic composition of the sugar monomers generated after methanolysis. The RG-I region of pectin is characterised by high contents of rhamnose, arabinose and galactose relative to the concentration of uronic acids. In addition to the monosaccharides that are characteristic for the RG-I region in pectin, minor amounts of other monosaccharides were also identified. These are considered to originate from remaining parts of the pectin and/or from non-pectin polysaccharides present in the raw material (Table 1).

TABLE 1 Monosaccharide composition of the NF after methanolysis (g/100 g).

		Batch number										
Parameter	**Unit**	**002**	**004**	**005**	**006**	**007**	**008**	**009**	**010**	**011**	**012**	**Analytical method**
Rhamnose	g/100 g	13.0	12.5	12.4	12.8	12.4	11.6	12.1	12.3	10.3	9.9	HPAEC–PAD, In house
Arabinose	g/100 g	25.0	25.8	24.8	25.8	25.5	25.6	30.7	31	24.8	22.9	HPAEC–PAD, In house
Galactose	g/100 g	16.5	17.8	15.9	18	16.8	16.4	15.7	17.2	13.3	14.4	HPAEC–PAD, In house
Uronic Acid	g/100 g	25.0	25.0	25.0	23.0	24.0	22.0	24.0	23.0	24.0	23.0	Spectrophotometric, In house
Glucose	g/100 g	0.8	0.8	1.1	0.9	0.8	0.7	1.0	1.0	0.8	1.3	HPAEC–PAD, In house
Fucose	g/100 g	0.6	0.6	0.7	0.6	0.6	0.6	0.5	0.5	0.4	0.4	HPAEC–PAD, In house
Xylose	g/100 g	0.6	0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4	HPAEC–PAD, In house
Mannose	g/100 g	0.5	0.4	0.6	0.5	0.5	0.4	0.5	0.5	0.4	0.4	HPAEC–PAD, In house
Total identified[1]		82.0	83.5	81.0	82.1	81.1	77.7	84.9	85.9	74.4	72.7	Calculation
Total typical for RG-I[2]		79.5	81.1	78.1	79.6	78.7	75.6	82.5	83.5	72.4	70.2	Calculation
Branched RG-I ratio[3]		31.9	34.9	32.8	34.2	34.1	36.2	38.3	39.2	37	37.7	Calculation

Abbreviation: HPAEC–PAD, high performance anion-exchange chromatography with pulsed amperometric detection.

[1]Sum of Rha, Ara, Gal, UA, Glc, Fuc, Xyl and Man.

[2]Sum of Rha, Ara, Gal, UA.

[3](Ara + Gal)/Rha.

3.3 | Production process

The NF is derived from carrot pomace, a secondary product resulting from the production of carrot juice or carrot concentrate. The original source material are carrots, which are commonly consumed as food in the EU. According to the information provided, the facility producing the NF is certified with FSSC 22000, ISO 14001 and ISO 50001. In the process of manufacturing the NF, the carrot pomace is first dispersed in a water-based medium. Subsequently, it undergoes incubation with a specific combination of enzymes under regulated conditions for a designated time [redacted] The enzymes in the mixture are tailored to selectively degrade the pectin portion within the carrot pomace solids. During this reaction, rhamnogalacturonan-I, a fragment of pectin and smaller carbohydrate units are liberated.

A pool of enzymes is used in the processing, [redacted] and [redacted] The [redacted] (Planas, 2000), [redacted]

The safety of the food enzyme containing [redacted] from the non-genetically modified [redacted] was evaluated by the [redacted]. The applicant provided a statement from the manufacturer of the enzyme preparation, declaring that application for the authorisation of the food enzyme containing [redacted] was submitted to the EC pursuing Regulation (EC) 1332/2008 [redacted].

After the hydrolysis step, the enzymes are inactivated by [redacted] Analytical data were provided to support the inactivation of enzymes. [redacted]

Remaining solids are separated from the liquid fraction containing the RG-I fraction by solid/liquid separation. [redacted] The retentate, which is used for further processing, is subject to heat treatment ([redacted]), drying and further subject to an additional pasteurisation ([redacted]) to ensure microbial quality. The concentrated cRG-I is spray-dried with no added ingredients, processing aids or additives, and packed in food-grade aluminium bags for storage.

The applicant indicates an estimated yield of production of NF from the carrot pomace of 70:1 (70 kg of carrot pomace to 1 kg NF). The applicant also estimated a yield of production of NF from fresh carrots of 500:1 (500 kg of fresh carrot to 1 kg NF).

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

3.4 | Compositional data

The NF consists of rhamnogalacturonan-rich pectin fraction (RG-I) from carrot.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain required characteristics, the applicant provided analytical information for 10 independent batches of the NF (Table 2). Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

TABLE 2 Batch to batch compositional analysis of the NF.

		Batch number										
Parameter	**Unit**	**002**	**004**	**005**	**006**	**007**	**008**	**009**	**010**	**011**	**012**	**Analytical method**
IDF*	% (w/w)	< 0.3	–	< 0.3	–	< 0.3	< 0.3	0.4	0.4	< 0.3	1.6	AOAC 991.43
SDF*	% (w/w)	65.0	–	71.0	–	70.9	70	61.4	63.6	59.9	59.6	AOAC 991.43
TDF*[a]	% (w/w)	65.0	–	71.0	–	70.9	70	61.8	64	59.9	61.2	AOAC 991.43
LMWDF*	% (w/w)	7.4	7.6	10.9	10	10.1	9.9	12.8	14.5	15.8	15.2	AOAC 2017.16
HMWDF* + RS	% (w/w)	68.7	73.4	62.8	72	73.5	69.3	67	69.2	60.9	63.9	AOAC 2017.16
TDF*[b]	% (w/w)	76.1	81	73.7	82	83.6	79.3	79.8	83.7	76.7	79.1	AOAC 2017.16
Protein (N* 6.25)	% (w/w)	2.2	1.6	2.7	2.2	1.8	2.0	1.5	1.7	1.8	2.1	Kjeldahl
Fat	% (w/w)	< 0.3	0.61	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	Soxhlet
Starch	% (w/w)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	0.5	0.6	< 0.3	< 0.3	Enzymatic-colorimetric - AOAC 2014.10

TABLE 2 (Continued)

Parameter	Unit	Batch number 002	004	005	006	007	008	009	010	011	012	Analytical method
Free sugars	% (w/w)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	1.3	HPAEC-PAD - ISO 22184
Ash	% (w/w)	3.37	2.89	3.34	3.19	3.47	5.02	2.99	3.03	3.22	3.39	Gravimetric (500-550°C)
Moisture	% (w/w)	3.14	5.16	4.25	3.19	3.58	4.08	3.98	3.99	4.18	3.70	Gravimetric

*The Panel interprets that 'dietary fibre' refers to non-digestible polysaccharides. Fibre is a non-digestible carbohydrate classified as dietary fibre by EFSA for the specific purpose of setting dietary reference values (DRV) for carbohydrates and dietary fibre (EFSA NDA Panel, 2010). The term 'fibre' is used as synonymous of non-digestible carbohydrates and does not reflect the additional requirement of having a beneficial physiological effect demonstrated by generally accepted scientific evidence laid down in Annex I of Regulation (EC) 1169/2011.

Abbreviations: IDF, insoluble dietary fibre; HMWDF + RS, high molecular weight dietary fibre + resistant starch; LMWDF, low molecular weight dietary fibre; RS, resistant starch; SDF, soluble dietary fibre; TDF, total dietary fibre; –, not analysed.

[a]TDF (IDF + SDF).

[b]TDF [LMWDF + (HMWDF + RS)].

The Panel noted that dietary fibres derived from pectin contain a high amount of bound galacturonic acid. It is known that such fibre components are underreported by using the method for the analysis of dietary fibre according to AOAC 2017.16, which is due to an incomplete precipitation in the 78% ethanol. The applicant provided proof of limitation of the applied total dietary fibre method. For the NF under assessment, this underreporting has been investigated and it is on average 7.6%. Considering the underreporting, the mass balance of the identified components of the NF ranges from 91.6 to 100.1% (average 96%). Considering the history of safe consumption of the source, the Panel considers that the proximate composition of the NF is sufficiently characterised.

The Panel notes variability of the concentrations of some proximate parameters, but this can be expected due to natural variability of the source material.

The applicant provided analytical data on minerals and heavy metals for 10 independently produced batches of the NF (Table 3).

TABLE 3 Batch to batch analysis of minerals, ions and heavy metals in the NF.

Parameter (unit)	Units	Batch number 002	004	005	006	007	008	009	010	011	012	Analytical method
Minerals and ions												
Sodium	mg/kg	890	790	750	860	840	1600	880	1000	1200	1500	ICP-MS
Magnesium	mg/kg	1200	1000	1100	1100	1200	2100	1500	1400	1600	1300	ICP-MS
Potassium	mg/kg	4500	5700	5600	6200	4400	4900	3400	3400	4500	5600	ICP-MS
Calcium	mg/kg	7400	5900	7400	6800	8100	13,000	7000	7000	7000	6800	ICP-MS
Phosphorous	mg/kg	240	190	270	340	240	220	250	220	310	480	ICP-MS
Nitrate	mg/kg	59.4	50.7	36.9	62.8	56.1	431	90.6	199	242	194	ASU L 26.00–1, 2018–10, LC-DAD
Copper	mg/kg	0.99	0.82	1.2	0.63	0.51	0.91	0.55	0.78	0.50	0.44	ICP-MS
Zinc	mg/kg	27	29	38	24	28	32	15	15	18	16	ICP-MS
Aluminium	mg/kg	< 0.8	1.1	< 0.8	0.89	0.94	1.4	3.1	5.8	9.8	13	ICP-MS
Boron	mg/kg	190	160	190	110	200	140	< 2.0	140	110	95	ICP-MS
Iron	mg/kg	27	28	31	26	27	27	14	48	63	85	ICP-MS
Manganese	mg/kg	17	20	33	15	17	25	0.47	16	16	16	ICP-MS
Nickel	mg/kg	0.14	0.16	0.19	0.15	0.14	0.32	< 0.08	0.14	0.28	0.19	ICP-MS
Selenium	mg/kg	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	0.44	< 0.04	< 0.04	< 0.04	ICP-MS
Heavy metals												ICP-MS
Arsenic	mg/kg	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	0.05	< 0.04	< 0.04	< 0.01	< 0.01	ICP-MS
Cadmium	mg/kg	0.60	0.55	0.95	0.51	0.64	0.75	0.10	0.10	0.09	0.11	ICP-MS
Mercury	µg/kg	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.5	0.5	ICP-MS
Lead	mg/kg	0.40	0.47	0.84	0.42	0.42	0.62	0.18	0.22	0.15	0.16	ICP-MS

Note: ICP-MS was performed as internal method.

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

In line with article 3 of Regulation (EU) 2023/915, the applicant provided experimentally determined processing factors of 39 for lead and 34 for cadmium to account for changes of the concentration of the contaminants caused by processing of fresh carrots to obtain the NF. They were taken into consideration when applying the maximum levels for lead and cadmium set out to fresh carrots in Annex I of Regulation 2023/915. By employing these processing factors to the results of the batch-to-batch analysis of the NF, it was confirmed that the heavy metal concentrations in the NF comply with the applicable maximum levels.

Analyses of mycotoxins, polycyclic aromatic hydrocarbons (PAH), dioxins and polychlorinated biphenyls (PCBs) were performed by the applicant and are reported in Appendix B. Values did not pose any concern.

Microbiological data on 10 independently produced batches of the NF are reported in Table 4. The Panel notes that the microbiological values of the analysed samples do not exceed the specification limits.

TABLE 4 Batch to batch microbiological analyses of the NF.

		Batch number										
Parameter (unit)	**Units**	**002**	**004**	**005**	**006**	**007**	**008**	**009**	**010**	**011**	**012**	**Analytical method**
Total plate counts	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100	100	< 100	< 100	Equal AFNOR 3M 01/01–09/89
Bacillus cereus	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	ISO 7932
Enterobacteriaceae	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	Equal AFNOR 3M 01/06–09/97
Escherichia coli	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Equal AFNOR 3M 01/08–06/01
Salmonella spp.	in 50 g	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	(AFNOR EGS 38/01–03/15)
Listeria monocytogenes	in 25 g	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	Equal AFNOR EGS 38/05–03/17
Sulphite reducing anaerobes*	cfu/g	< 10	–	< 10	–	< 10	–	–	< 10	< 10	–	ISO 15213:2003
Yeast & moulds	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Equal AFNOR 3M 01/13–07/14

Abbreviation: n.d., not detected.

*Includes *Clostridium* spp.

Due to the nature of the novel food and considering the applied production process, its consumption is not expected to lead to exposure to small particles different from those of natural origin present in similar matrices of plant origin and considered safe for consumption.

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

3.4.1 | Stability

The applicant performed stability tests with five independently produced batches of the NF. The tests were carried out at normal storage conditions at 18°C and at 50% RH for up to 2 years. The batches were analysed for physical and microbiological parameters. In addition, the molecular weight distribution was analysed at different time points between 1 and 2 years and compared with the initial distribution of the respective batch (Table 5).

The applicant provided the analytical methods used and the respective certificates of analyses.

All the batches tested confirmed the stability of the NF up to the latest time point tested, and results complied with the specifications of the NF.

The polymeric structure of the NF showed no changes during the stability tests, indicating no degradation of the polysaccharide structure.

TABLE 5 Physical, microbiological and molecular stability of the novel food.

Parameter	Units	Time of storage (months)									
Physical		**0**	**0**	**0**	**0**	**0**	**24**	**24**	**24**	**30**	**24**
BatchID		002	005	007	010	011	002	005	007	010	011
Moisture	g/100 g	3.1	4.3	3.6	4.0	4.2	5.3	6.5	5.5	4.6	5.5
Water activity		0.11	0.09	0.07	0.08	0.07	0.12	0.19	0.14	0.13	0.19
Microbiological											
Total aerobic plate count	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100
Yeast	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Moulds	cfu/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Bacillus cereus	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100
Enterobacteriaceae	cfu/g	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100
Salmonella spp.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Escherichia coli		< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Molecular mass fractions (%)	Mw range (kDa)										
I	> 910	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
II	127–910	12.7	7.4	12.6	9.3	8.5	12.3	10.9	12.2	9.1	7.7
III	11.4–127	47.3	43.2	46.5	50.7	48.2	48.3	52.6	47.2	50.3	46.8
IV	1.9–11.4	34.1	40.0	34.6	35.5	36.8	35.1	32.6	34.8	34.9	37.8
V	< 1.9	5.9	9.4	6.4	4.5	6.4	4.3	3.9	5.6	5.8	8.0

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

3.5 | Specifications

The specifications of the NF are indicated in Table 6.

TABLE 6 Specifications of the NF.

		Description: cRG-I is an enzymatic extract of carrot pomace enriched for rhamnogalacturonan-I domains (RG-I). Source: *Daucus carota L., subspecies Sativus*
Physical and chemical properties	**Unit**	**Specification**
Appearance	–	Free flowing
Colour	–	Off-white to beige
Odour/taste	–	Neutral
Solubility	–	Soluble in water
Water activity	–	< 0.6
Composition		
Moisture	g/100 g	≤ 7
Free sugars	g/100 g	≤ 3
Soluble dietary fibre	g/100 g	≥ 60
Total dietary fibre	g/100 g	≥ 70
Crude protein	g/100 g	≤ 4
Ash	g/100 g	≤ 5
Fat	g/100 g	≤ 2
Contaminants		
Lead	mg/kg	≤ 1.0
Cadmium	mg/kg	≤ 1.0

(Continues)

TABLE 6 (Continued)

Physical and chemical properties	Unit	Description: cRG-I is an enzymatic extract of carrot pomace enriched for rhamnogalacturonan-I domains (RG-I). Source: *Daucus carota L., subspecies Sativus* Specification
Arsenic	mg/kg	≤ 0.1
Mercury	mg/kg	≤ 0.1
Perchlorate	mg/kg	≤ 0.05
Microbiological		
TAMC	CFU/g	< 1000
TYMC	CFU/g	< 100
Bacillus cereus	CFU/g	< 100
Enterobacteriaceae	CFU/g	< 100
Escherichia coli	CFU/g	< 10
Salmonella spp.		Not detected in 25 g
Listeria monocytogenes		Not detected in 25 g
Sulphite reducing anaerobes	CFU/g	< 30

Abbreviations: CFU, colony forming units; Soluble HMWDF, high molecular weight dietary fibre; TAMC, total aerobic microbial count; TYMC, total yeast and mould count.

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

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

There is no history of use of the NF.

The source of the NF is the root vegetable carrot (*Daucus carota L.*). Carrot is largely consumed in Europe and outside Europe with a very long history of use. Pectin, the substrate for cRG-I manufacturing, has been widely used in food production due to its functionality as an emulsifier, gelling agent and stabiliser, among others. The structure and properties of pectin vary based on the plant source and manufacturing process. Rhamnogalacturonan-I (RG-I), a structural element of pectin in plants such as carrots, has been consumed for centuries in Europe. RG-I has been identified and isolated from various food sources including bell pepper, carrot (McKay et al., 2021), tomato, aubergine, strawberry, apple (Cornuault et al., 2018), pumpkin, pomegranate, potato (Thorning et al., 2020) and watermelon (Trandel et al., 2021). However, the structure and composition of RG-I is species-specific and can be affected by processing conditions.

RG-I structures are constituents of various processed fruit and vegetable products and are released into foodstuffs due to enzymatic hydrolysis of pectin. They can be found in fruit and vegetable juices and purees, and are structurally similar to those in cRG-I.

The applicant conducted systematic literature research on isolated RG-I structures with potential use as ingredients.

Retrieved articles reported the identification and isolation of RG-I like pectic structures in fruits or vegetables and studied the impact of technological processes on the structure–function relationships of pectin, or the prebiotic potential of pectin and RG-I. However, none of the articles reported the specific use of isolated or enriched RG-I as food ingredient outside scientific investigations.

3.7 | Proposed uses and use levels and anticipated intake

3.7.1 | Target population

The target population for the NF proposed by the applicant varies by intended use:

- As ingredient in several food products: General population.
- As ingredient in food for special medical purpose (FSMP); in meal replacement for weight control (MRWC): General population excluding infants.
- As food supplement (FS): General population excluding infants.
- As ingredient in total diet replacement for weight control (TDRWC): Adult population.

3.7.2 | Proposed uses and use levels

The NF is proposed to be used as an ingredient in several food products. Food products are defined using the FoodEx2[7] hierarchy, and the maximum use levels are reported in Table 7.

For FS. The applicant intends to market the NF as defined in Directive 2002/46/EC at a maximum daily intake of 750 mg/day for young children (1 to < 3 year of age), and 1500 mg/day for individuals of 3 year of age and above.

For FSMP. The applicant intends to market the NF in accordance with the nutritional requirements of the persons for whom the products are intended according to Regulation (EU) No 609/2013. The applicant proposed a maximum daily intake of 750 mg/day for young children (1 to < 3 year of age) and 1500 mg/day for individuals of 3 year of age and above.

For TDRWC. The applicant intends to market the NF as ingredient in accordance with Regulation (EU) No 609/2013, for adults only, at a maximum daily intake of 1500 mg/day.

For MRWC. The applicant intends to market the NF as ingredient at a dose of 250 mg/meal for young children (1 to < 3 year of age), and at 500 mg/meal for individuals of 3 year of age and above.

TABLE 7 Food categories and maximum use levels intended by the applicant.

FoodEx2 level	FoodEx2 code	Food category	Max use level (mg NF/100 g)
2	A03RC	Ready-to-eat meal for infants and young children	450
3	A00EJ	Muesli and similar mixed breakfast cereals	5000
3	A00EY	Cereal bars	3750
3	A03BB	Fruit nectars (min. 25%–50% fruit as defined in EU legislation)	500
3	A03DB	Mixed fruit and vegetable juices	500
3	A03DE	Mixed juices with added ingredients	500
3	A03RA	Biscuits, rusks and cookies for children	3750
3	A03RN	Fruit and vegetable juices and nectars specific for infants and young children	625
3	A03RP	Special food for children's growth	375
3	A041L	Soups (ready-to-eat)	750
3	A04PQ	Vegetable juices	500
3	A0B9J	Soups (dry mixture uncooked)	5000
4	A02MP	Flavoured milks	750
4	A02NQ	Yogurt drinks, including sweetened and/or flavoured variants	750
4	A03TH	Milk imitates	750
5	A03TR	Imitation cream	10,000
5	A03TV	Soy yogurt	1200
5	A03TY	Imitation cheese	3750
5	A03TZ	Imitation yogurt, non soy	1200

3.7.3 | Anticipated intake of the NF as food ingredient

EFSA performed an intake assessment of the anticipated daily intake of the NF based on the applicant's proposed uses and maximum proposed use levels (Table 7), using the EFSA Dietary Exposure (DietEx) Tool[8], which is based on individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile estimated daily intakes of the NF (on a mg/kg body weight (bw) basis), among the EU dietary surveys, are presented in Table 8.

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).

[7]FoodEx2 is an EFSA standardised food classification and description system https://www.efsa.europa.eu/en/data/data-standardisation.
[8]https://www.efsa.europa.eu/it/science/tools-and-resources/dietex.

TABLE 8 Intake estimate of the NF resulting from its use as an ingredient in the intended food categories indicated in Table 7 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	0.3	136.8	0.0	353.9
Young children[c]	1–< 3	5.9	63.0	35.7	247.7
Other children	3–< 10	6.1	47.5	36.3	157.8
Adolescents	10–< 18	2.0	19.3	16.2	77.0
Adults[d]	≥ 18	0.6	15.3	2.9	51.7

[a]Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 13/3/2024. 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 13/3/2024. The lowest and the highest P95 observed among all EU surveys are reported in these columns (P95 based on less than 60 individuals are not considered).

[c]Referred as 'toddlers' in the EFSA food consumption comprehensive database (EFSA, 2011).

[d]Includes elderly, very elderly, pregnant and lactating women.

3.7.4 | Anticipated intake of the NF as food supplement

The applicant proposed a maximum daily intake of 750 mg/day for young children (1 to < 3 years of age), and 1500 mg/day for individuals of 3 years of age and above. The intake of the NF used as FS is reported in Table 9.

TABLE 9 Intake level of the NF as food supplement.

Population group	Age (years)	Body weight[a] (kg)	Use level (mg/day)	Intake (mg/kg bw per day)[b]
Infants	< 1	5	–	–
Young children	1 to < 3	12	750	62.5
Other children	3 to < 10	23	1500	64.9
Young adolescents	10 to < 14	43	1500	34.9
Old adolescents	14 to < 18	61	1500	24.5
Adults	≥ 18	70	1500	21.4

[a]Default and average body weights for each population group are available in EFSA Scientific committee (2012).

[b]Intake in 'mg/kg bw per day' are calculated by considering the use levels in 'mg/d' and default body weights defined in EFSA Scientific committee (2012).

3.7.5 | Combined intake of the NF from its use as ingredient and other forms

The total anticipated intake of the NF from used as a food ingredient and FS is reported in Table 10. The Panel notes that the combined intake estimates of the NF as 'TDRWC' and 'FS' would be lower than the estimated intake of the NF used as food ingredient and FS.

TABLE 10 Total intake of the NF resulting from its uses as an ingredient and as a food supplement.

Population group	Age (years)	Body weight[a] (kg)	Highest[b] P95 intake from the NF used as ingredient (mg/kg bw per day)	Intake from the NF used as a food supplement (mg/kg bw per day)[c]	Total intake[d] (mg/kg bw per day)
Infants	< 1	5	353.9	–	353.9
Young children	1 to < 3	12	247.7	62.5	310.2
Other children	3 to < 10	23	157.8	64.9	222.7
Adolescents	10 to < 18	43	77.0	34.9[e]	111.9[e]
Adults	≥ 18	70	51.7[f]	21.4	73.1

[a]Default and average body weights are defined in EFSA Scientific Committee (2012).

[b]Intakes are assessed for all EU dietary surveys available in the food comprehensive database. The highest P95 observed among all surveys is reported in this column (P95 calculated based on less than 60 individuals are not considered).

[c]Intake in 'mg/kg bw per day' are calculated by considering the use levels in 'mg/d' and default body weights defined in EFSA (2012).

[d]Total intake is the sum of the intake from NF ingredient use (highest P95) and from the NF used as a food supplement, for each population group.

[e]Intakes are assessed separately for young (10–14 years) and old adolescent (14–18 years); the maximum intake among these two sub-populations is reported here.

[f]Intakes are assessed separately for adults (18–64 years), elderly (65–74 years) and very elderly (≥ 75 years); the maximum intake among these three sub-populations is reported here.

3.7.6 | Combined intake of cRG-I from the NF and other sources

The intake of cRG-I as NF should be combined with the intake of RG-I structures from pectin as a food additive and from natural sources to fully cover all possible sources of intake of RG-I like structures.

Generally, the Rhamnogalacturonan-I domain is considered to constitute 20%–35% of total pectin in the plant cell wall (Kaczmarska et al., 2023).

However, due to scarce and highly variable data of RG-I concentration, robust estimates are not possible.

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

As described in Section 3.4 Compositional data, the NF consists of indigestible soluble polysaccharides derived from pectin.

No ADME study has been performed in animals or humans. In order to confirm that the NF is subject to colonic metabolism, the applicant conducted a series of *in vitro* fermentation experiments with pilot material of cRG-I (test item representative for the NF), using human faecal microbiota (Van den Abbeele et al., 2020; Van den Abbeele et al., 2021). According to the applicant, the *in vitro* fermentation experiments could demonstrate that the NF is fermented in model systems for the human gut using the SHIME® Model, increasing the abundance of some *Bacteroidetes* and Bifidobacteria species and triggering the production of short-chain fatty acids (SCFA). The literature provided by the applicant confirmed the formation of SCFA resulting from the microbial fermentation of the test item under conditions simulating the colon, which is typical for the fermentation of non-digestible carbohydrates (NDC). (Desai et al., 2024; Van den Abbeele et al., 2020; Van den Abbeele et al., 2021; Van den Abbeele et al., 2023).

The information provided by the applicant suggests that cRG-I, the main compound of the NF, follows the fate of the non-starch polysaccharide pectins and of other non-digestible carbohydrates, i.e. not digested by human's or rat's enzymes in the upper gastrointestinal tract, but subject to microbial fermentation in the large intestine, in rats in particularly in the caecum.

The Panel notes that the findings of the 90-day study with the NF, i.e. the adaptive effects of increased size and weight of the caecum and proliferation of the caecal mucosa (see Section 3.12 Repeated dose toxicity studies) indicate that SCFAs are also formed in the rat model as a result of microbial fermentation of the NF.

The Panel considers that no further ADME testing is necessary for the safety assessment of the NF.

3.9 | Nutritional information

The applicant provided a nutritional analysis of the NF. The specifications proposed by the applicant indicate that the NF may contain ≥ 70% dietary fibre, ≤ 4% protein and ≤ 2% fat. The mean concentration of total dietary fibre in 10 batches is 79.5%. The Panel notes that, based on the highest concentrations of fibre in the NF (Table 2) and combined estimated intakes from all uses of the NF (Table 10), the fibre intake from the NF ranges between 1.77 g/day (in infants) to 5.12 g/day (in other children and adults). From a nutritional point of view, dietary fibre is defined as non-digestible carbohydrates plus lignin. Non-starch polysaccharides with a degree of polymerisation > 9 are a type of dietary fibre which includes pectins. Dietary Reference Values for total (DRVs) dietary fibre have been established, i.e. 25 g/day in adults and intake of 2 g/MJ in children from 1 year (EFSA NDA Panel, 2010). Average intakes of dietary fibre in national dietary surveys across European countries are generally below the DRVs (EFSA NDA Panel, 2022).

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

3.10 | Toxicological information

The applicant provided several toxicological studies on the NF, which were conducted in compliance with Organisation for Economic Co-operation and Development (OECD) principles of GLP and in accordance with the OECD test guidelines No 471, 487 and 408 from the (OECD, 2018, 2020, 2023).

These studies which were claimed proprietary by the applicant are listed in Table 11.

TABLE 11 List of toxicological studies with the NF.

References	Type of study	Test system	Dose
Study No. AME02002 (Unpublished, 2022a)	Bacterial reverse mutation test (GLP, OECD TG 471)	*S.* Typhimurium LT2 Strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2 Strain *uvrA*	Up to 5000 µg/plate (absence and presence of S9 mix)
Study No. 192-471-8431 (Unpublished, 2024)	Bacterial reverse mutation test (GLP, OECD TG 471) performed with four additional batches	*S.* Typhimurium LT2 Strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2 Strain *uvrA*	Up to 5000 µg/plate (absence and presence of S9 mix)
Study No. MNT02016 (Unpublished, 2022b)	*In vitro* Mammalian Cell Micronucleus Test (GLP, OECD TG 487)	Human lymphocytes, isolated from fresh whole human blood	Control: 0 (solvent, Type 1 Water); 2222, 3333 and 5000 µg/mL in absence and presence of metabolic activation for 3 h and 987.47, 1481 and 2222 µg/mL in absence of metabolic activation 24 h (continuous treatment schedule)
Study No. 20155854 (Unpublished, 2019a)	90-day repeated dose oral toxicity study with a 14-day recovery period (GLP, OECD TG 408)	Wistar rats	0%, 2.5%, 5% and 10% dietary inclusion level in food, corresponding to 0, 1752, 3396, 6907 mg/kg bw per day in males and 0, 1923, 3916 and 7753 mg/kg bw per day in females, based on food consumption
Other			
Study No. 20151914 (Unpublished, 2019b)	14-Day Dose Range Finding study	Wistar rats	0.5%, 7.5%, 10% (% w/w)

3.11 | Genotoxicity

The applicant performed a battery of *in vitro* genotoxicity tests with the NF.

One batch of cRG-I was tested for mutagenic activity using genetically modified Salmonella Typhimurium LT2 bacteria of strains TA1535, TA1537, TA98 and TA100, and *Escherichia coli* WP2 strain uvrA as indicator organisms, in compliance with OECD Test Guideline 471 (2020).

The Ames assay was conducted using the plate incorporation method for all five indicator strains in both the presence and absence of an *in vitro* metabolic activation system based on the S9 fraction obtained from Aroclor 1254-induced rat livers (S9 mix). The dose range tested was 1.6 to 5000 µg per plate. As the result was considered negative, a confirmatory test was carried out using the liquid pre-incubation method (Test 2) with the same dose range of 1.6 to 5000 µg per plate in the presence and absence of S9 mix. The test item showed evidence of cytotoxicity at 5000 µg per plate. Precipitate was not seen. The maximum dose level scored for revertant colonies was 5000 µg per plate. No significant increase in numbers of revertant colonies was seen with any of the five indicator strains either in the presence or absence of S9 mix. It was concluded that cRG-I was not mutagenic for *S.* Typhimurium LT2 strains TA1535, TA1537, TA98 and TA100, and WP2 strain uvrA, either in the presence or absence of S9 mix, when tested under the conditions used in this study (Unpublished, 2022a).

Considering the nature of the NF (obtained from a by-product), the applicant was requested to perform the bacterial reverse mutation test on four additional batches. The dose range of the NF tested was 50 to 5000 µg per plate. The test items (additional four batches) did not induce gene mutations in the genome of the strains of *S.* Typhimurium TA98, TA100, TA1535 and TA1537 and of *E. coli* WP2 uvrA (Unpublished, 2024).

In addition, cRG-I was tested for its potential to induce micronucleus formation in the *in vitro* micronucleus test using isolated human lymphocytes. The cells were treated in presence and absence of S9 for 3 h and without S9 for 24 h in accordance with the OECD Guideline 487 (2016). The concentration tested corresponded to 2222, 3333 and 5000 µg/mL for short term treatment and from 987.7, 1481 and 2222 µg/mL for the continuous treatment. No precipitate was observed while the cytotoxicity detected at the highest concentration tested corresponded to 8% (3 h + S9), 25% (3 h -S9) and 55% (24 h -S9). The concurrent positive controls produced statistically significant increases in micronuclei compared with the concurrent negative controls. Treatment with the NF did not induce significant increases in micronucleus formation at all conditions applied (Unpublished, 2022b).

Taking into account the test results provided the Panel considers that there are no concerns regarding genotoxicity.

3.12 | Repeated dose toxicity studies

The applicant provided an in vivo 14-day range finding study by dietary administration in Wistar male rats (Unpublished, 2019b). The administration of the NF for 14 days at dietary levels up to 10% (w/w) was well tolerated.

Therefore, the applicant selected the dose levels of 0 (control), 2.5, 5.0 and 10.0% (w/w) for the subsequent sub-chronic (90-day) oral toxicity study in Wistar rats.

The 90-day oral toxicity study (Unpublished, 2019a) was performed following the OECD Principles of GLP and according to OECD Test Guideline No. 408 (OECD, 2018. The study was performed with four groups of 20 Wistar rats (10 males and 10 females in each group) given experimental diets containing 0, 2.5, 5 or 10% of the NF, corresponding to 0, 1752, 3396, 6907 mg/kg bw per day in males and 0, 1923, 3916 and 7753 mg/kg bw per day in females, based on food consumption. The test item was added to standard rodent diet at the expense of added starch. The controls received 10% starch.

A few endpoints were showing statistically significant dose-related changes (water consumption in male rats, mean fore limb grip strength in male rats, absolute and relative caecal weight in male and female rats, absolute thymus weight in male rats, relative kidney weight in female rats), suggesting an effect of the test item administered.

Statistically significantly increased water consumption of male rats at mid and high dose were seen in weeks 1 and 2 of the study. The highest statistically significant increase in water consumption was +29%. This effect was not observed in female rats.

Mean fore limb grip strength tested within the functional observation battery was statistically significantly lower in males in the mid dose and high-dose group compared to controls (−40% and −44%, respectively). A dose-dependent trend was shown in mean fore limb grip strength and mean hind limb grip strength (−15% in mid dose males and −22% in high-dose males), although this effect was not statistically significant. No dose-related effect on mean fore limb or hind limb grip strength was observed in female rats. Since these were isolated data, in males only, within the historical controls, they were not considered toxicologically relevant.

Statistically significantly increased absolute caecum weights (with or without content) were observed in male rats at mid and high dose (+44%/+36% and +52%/+50%, respectively) and in female rats at high dose (+36%/+27%). While male rats showed also statistically significantly increased relative caecum weight (full and empty) at mid dose (+37%/+29%) and high dose (+52%/+50%), a statistically significant increased caecum weight (full and empty) was observed in female rats only at high dose (+41%/+26%). No test item-related macroscopic changes were seen at necropsy. In three female rats minimal hypertrophy of the caecal mucosa was observed at high dose. This effect was not observed in male rats. There are indications that the intake of high doses of low- or indigestible carbohydrates by rats leads to the formation of SCFA in the large intestine, which is considered to be related to epithelial cell proliferation (Xiao et al., 2015). Caecal enlargement and hypertrophy of the caecal mucosa is a frequently observed effects in rats after ingestion of high doses of low- or indigestible carbohydrates due to their microbial fermentation, typically associated with the formation of SCFA, which is considered to be not toxicologically relevant for humans (EFSA ANS Panel, 2017; EFSA NDA Panel, 2010; Xiao et al., 2015). Due to the low intensity of the minimal hypertrophy of the caecal mucosa observed at high dose in three female rats, the Panel considers those as non-adverse and like the caecum enlargement rather an adaptive effect. Minimal (grade 1) lymphogranulocytic inflammatory infiltrates in the caecal mucosa were observed in the caecum of two high dosed male rats. Such findings were not reported for female rats, who exhibited caecum enlargement to a lesser degree. Since cell infiltrates were only seen locally and no changes in white blood cells indicating absence of systemic response were seen, the Panel considers this finding to be an indication for a mild perturbation of the caecal homeostasis causally related to the high dose of the indigestible carbohydrates.

Statistically significantly increased relative kidney weight was observed in female rats at high dose (+12%) with a dose-dependent trend at lower doses (+2%, +5%), while no effects on kidney weight were observed in males. Increase in kidney weight is considered to be a test item-related effect, often associated with high doses of low- or indigestible carbohydrates, which lead to an increased bioavailability of minerals and subsequently to an adaptation of the kidneys (increase of kidney function) in rats in order to cope with the higher load of minerals (Xiao et al., 2015). However, increased water consumption in the first few weeks of the study was observed only in males, but not in females, and no changes in clinical parameters were recorded. No urinalysis was performed that would substantiate the interpretation. No treatment-related macroscopic changes were seen on kidneys at necropsy. There were no histopathological changes observed in kidneys. Therefore, the Panel considers that the observed increase in relative kidney weight < 15% in high-dose females without histopathological correlates is an adaptive rather than an adverse effect.

Overall, the Panel considers that reported effects on the caecum and kidney are causally related to the test item but are of adaptive nature and toxicologically not relevant for human. The Panel also notes that such effects on caecum and kidney weight were also reported in the two 90-day rat studies with pectin-derived oligosaccharides assessed by the EFSA ANS Panel (2017). The Panel considers that the provided 90-day study did not show effects of toxicological relevance for humans up to the highest dose tested.

3.13 | Human data

The Panel notes that there are no human studies conducted with the NF. However, the applicant performed a literature search to identify human safety studies on cRG-I or RG-I-like pectin structures. Three publications were identified that described human clinical studies with rhamnogalacturonan-enriched pectic polysaccharides (Lutter et al., 2021; McKay et al., 2021; McKay et al., 2022). The Panel notes that those studies were primarily designed to investigate beneficial effects (immune responses, immunomodulation) and that these publications do not report on the safety of the NF.

3.14 | Allergenicity

The NF contains < 4% protein by weight. The most important allergenic protein in carrot is Dau c 1. Dau c 1 has been reported to be heat-labile and to denature at temperatures above 43°C (Lyons et al., 2018). Considering the production process of the NF (see Section 3.3 Production process), which includes heat treatments up to 90°C, Dau c 1 may have been inactivated or denatured. However, the effect of processing on the allergenicity of foods (i.e. IgE-binding capacity and clinical reactivity) is difficult to predict (EFSA NDA Panel, 2014), and no allergenicity studies with the NF have been submitted.

The Panel considers that the NF may retain the allergenic potential of carrots and that allergic reactions to the NF may occur, but they will not be dissimilar from those triggered by the consumption of carrots.

4 | DISCUSSION

The NF, which is the subject of the application, is cRG-I, a rhamnogalacturonan-rich polysaccharide fraction derived from carrot pomace. The novel food is intended for use as ingredient in various food products targeting the general population, in food for special medical purposes, meal replacement for weight control and food supplements targeting the general population excluding infants, and in total diet replacement for weight control targeting the adult population.

Intake estimates for the NF consumed via foods to which it could be added as an ingredient were performed for the general population, based on the EFSA Comprehensive European Food Consumption Database. The highest intake estimate was calculated for infants at 353.9 mg NF/kg bw per day at the 95th percentile.

Based on the data provided, the Panel considers that there are no concerns regarding genotoxicity. The repeated dose toxicity studies in rats did not raise safety concerns up to the highest dose tested, i.e. 7753 mg NF/kg body weight per day. The Panel notes that the margin of exposure between that highest dose received by rats in the 90-day study and the highest (95th percentile) intake estimates for the NF at the proposed uses (including FS), ranges from 22 (for infants) to 106 (for adults). The Panel considers the MoE to be sufficient taking into consideration that the source (i.e. carrots), nature, composition and production of the NF do not raise safety concerns.

5 | CONCLUSIONS

The Panel concludes that the NF, cRG-I, a rhamnogalacturonan-rich polysaccharide fraction derived from carrot pomace, is safe under the proposed conditions of use.

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

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant as listed in Appendix C.

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
AFNO	Association Française de Normalisation (French Association for Standardisation)
AME	alternariol monomethylether
AOAC	Association of Official Agricultural Chemists
bw	body weight
CFU	colony forming units
cRG-I	rhamnogalacturonan-I enriched carrot fibre
DRV	dietary reference values
FS	food supplement
FSMP	food for special medical purpose
FSSC	Food Safety System Certification
GalA	D-galacturonic acid
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HG	homogalacturonan
HMWDF + RS	high molecular weight dietary fibre + resistant starch.
HPAEC–PAD	high performance anion-exchange chromatography with pulsed amperometric detection
HPSEC–RI	high performance size exclusion chromatography with refractive index detection
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDF	insoluble dietary fibre

ISO	International Organization for Standardization
kDa	kiloDalton
L-Araf	L-arabinofuranose
LMWDF	low molecular weight dietary fibre
MRWC	meal replacement for weight control
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NDC	non-digestible carbohydrates
NF	novel food
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PAH	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PDI	polydispersity index
RG-I	rhamnogalacturonan-I
RH	Relative Humidity
RS	resistant starc
SCFA	short-chain fatty acids
SDF	soluble dietary fibre
TAMC	total aerobic microbial count
TDF	total dietary fibre
TDRWC	total diet replacement for weight control
TEA	tenuazonic acid
TYMC	total yeast and mould count

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2023-00025

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SUPPORTING INFORMATION

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

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APPENDIX A

Molecular weight distribution (high and low Mw part) derived from HP-SEC profiles (*N* = 10). Molecular weight distribution derived from HP-SEC profiles (*N* = 10), Mw ranges based on pullulan [kDa]

MW	Units	# 002	# 004	# 005	# 006	# 007	# 008	# 009	# 010	# 011	# 012
High MW part	% (w/w)	54	61	45	55	53	53	50	53	50	47
Mn	(g/mol)	41,805	38,967	37,344	40,557	41,200	39,324	40,256	39,293	39,026	40,272
Mw	(g/mol)	85,027	80,889	71,506	80,337	83,380	77,675	78,022	75,875	76,077	75,987
PDI		2	2.1	1.9	2.0	2.0	2.0	1.9	1.9	1.9	1.9
Peak at 10 min		52,099	51,554	49,485	51,148	51,952	52,211	50,383	48,328	47,647	47,758
Low MW part	% (w/w)	36	29	42	34	36	36	38	37	41	35
Mn	(g/mol)	3175	3516	2803	3106	3052	3080	3286	3710	3260	2915
Mw	(g/mol)	4723	5022	4426	4691	4609	4709	5268	5555	5155	5039
PDI		1.5	1.4	1.6	1.5	1.5	1.5	1.6	1.5	1.6	1.7
M peak		3849	3813	3751	3619	3647	3749	4004	4049	3833	3896
Salt and sugar	% (w/w)	1	1	2	1	1	1	1	1	1	3
Average molecular weight and polydispersity index											
Polymer peak	Mass %	91	90	87	89	89	87	88	90	91	82
Salt and sugar	Mass %	1	1	2	1	1	1	1	1	1	3
Polymer peak Mn	g/mol	7103	9136	5349	7271	6815	7005	6848	7913	6583	6196
Polymer peak Mw	g/mol	52,834	56,384	38,992	51,667	51,626	49,114	46,604	46,854	44,268	45,585
PDI		7.4	6.2	7.3	7.1	7.6	7	6.8	5.9	6.7	7.4
HPSEC area (%) (Mw based on pullulan (kDa))											
Fraction I: > 910	kDa	0	0	0	0	0	0	0	0	0.1	0
Fraction II: 127–910	kDa	12.7	11.5	7.4	11	12.6	10.7	9.6	9.3	8.5	8.6
Fraction III: 11.4–127	kDa	47.3	54.8	43.2	47.2	46.5	49.2	48.9	50.7	48.2	48.2
Fraction IV: 1.9–11.4	kDa	34.1	30.2	40.0	35.9	34.6	34.0	35.2	35.5	36.8	33.4
Fraction V: < 1.9	kDa	5.9	3.4	9.4	6	6.4	6.1	6.2	4.5	6.4	9.8

Abbreviations: Mn, number average molecular mass; Mw, weight average molecular mass; PDI, polydispersity index (Mw/Mn), M at 10 min, Mw at Rt 10 min; M peak, mean Mw.

APPENDIX B

Batch to batch analyses of mycotoxins, contaminants, pesticide residue in the NF

Parameter (Unit)	Units	Batch number										Analytical method
		002	004	005	006	007	008	009	010	011	012	
Mycotoxin												LC–MS/MS
Aflatoxin B1	µg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS
Aflatoxin B2	µg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS
Aflatoxin G1	µg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS
Aflatoxin G2	µg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	LC–MS/MS
Aflatoxin total	µg/kg	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	LC–MS/MS
Alternaria toxins – alternariol	µg/kg	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	LC–MS/MS
Altenuene (ALT)	µg/kg	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	LC–MS/MS
Alternariol Monomethylether (AME)	µg/kg	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 2	LC–MS/MS
Tenuazonic acid (TEA)	µg/kg	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	LC–MS/MS
Tentoxin (TEN)	µg/kg	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	LC–MS/MS
Patulin	µg/kg	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	< 20	LC–MS/MS
Ochratoxin A	µg/kg	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	LC–MS/MS
Contaminants												
PAH4; Benz(a) an- trhracene		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	GC–MS/MS
PAH4; Benzo(a)pyrene		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	GC–MS/MS
PAH4; Benzo(b) fluoranthene		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	GC–MS/MS
PAH4; Chrysene		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	GC–MS/MS
Sum PAH4		< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	calculated
WHO (2005)- PCDD/F+PCB TEQ (upper bound)	pg/g wet weight	0.0873	0.074	0.069	0.066	0.065	0.115	0.118	0.119	0.116	0.12	Conform EC 2017/644 (food) and EC 2017/771 (feed), Calculation
Quantitative multi pesticide screening												
Other screened pesticides	mg/kg	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	Internal method, LC–MS/MS

APPENDIX C

List of elements of the dossier for which data protection was requested

NF Section	Elements of the application dossier for which a request for data protection was filed by the applicant	Considered as pertinent for the NDA Panel to reach the conclusion on the safety (Yes/No)
Identity	NF-2022-7430 2.2 Identity UPDATE 20240611 CONFIDENTIAL Annex 2.2.01_HPSEC_cRG-I_batches 02-05 CONFIDENTIAL Annex 2.2.02_HPSEC_cRG-I_batches 06-08 CONFIDENTIAL Annex 2.2.03_HPSEC_cRG-I_batches 09-12 CONFIDENTIAL	Yes
Compositional	NF2022-7430 2.4. Compositional data cRG-I UPDATE 20250107 CONFIDENTIAL Annex 2.4.04_Summary compositional data cRG-I UPDATE 20240611 CONFIDENTIAL Annex 2.4.05_CoA batch 002 CONFIDENTIAL.pdf Annex 2.4.06_CoA batch 004 CONFIDENTIAL.pdf Annex 2.4.07_CoA batch 005 CONFIDENTIAL.pdf Annex 2.4.08_CoA batch 006 CONFIDENTIAL.pdf Annex 2.4.09_CoA batch 007 CONFIDENTIAL.pdf Annex 2.4.10_CoA batch 008 CONFIDENTIAL.pdf Annex 2.4.1 1_CoA batch 009 CONFIDENTIAL.pdf Annex 2.4.12_CoA batch 010 CONFIDENTIAL.pdf Annex 2.4.13_CoA batch 011 CONFIDENTIAL.pdf Annex 2.4.14_CoA batch 012 CONFIDENTIAL.pdf Annex 2.4.16 stability data CoAs T1 CONFIDENTIAL Annex 2.4.17 stability data CoAs T2 update 20230323 CONFIDENTIAL Annex 2.4.24_Methanolysis_cRG-I_batches 02-05 update 20230323 CONFIDENTIAL Annex 2.4.25_Methanolysis_cRG-I_batches 06-08 update 20230323 CONFIDENTIAL Annex 2.4.26_Methanolysis_cRG-I_batches 09-012 update 20230323 CONFIDENTIAL Annex 2.4.27 stability data CoAs T3 CONFIDENTIAL Annex 2.4.28_CoA stability T4 tested batch 011 CONFIDENTIAL Annex 2.4.29 stability T5 all tested batches 20230920 PUBLIC Annex 2.4.30_CoA Luff batches 8-12 CONFIDENTIAL Annex 2.4.42_CoA stability data T6 CONFIDENTIAL	Yes
Toxicological information	NF-2022-7430 Section 2.10. Toxicity information CONFIDENTIAL 20221214.pdf NF-2022-7430 2.10.3.1 Genotoxicity UPDATE 20250107 CONFIDENTIAL NF-2022-7430 2.10.3.2 Sub-chronic oral toxicity UPDATE 20240611 CONFIDENTIAL Annex 2.10.03 Ames assay CONFIDENTIAL Annex 2.10.04 MnVit Human Lymphocytes CONFIDENTIAL.pdf Annex 2.10.05 Dose-range finding study for OECD 408 CONFIDENTIAL.pdf Annex 2.10.06 90 days oral toxicity study CONFIDENTIAL.pdf Annex 2.10.07 Comparison pilot and commercial scale cRG-I CONFIDENTIAL 20221216 Annex 2.10.10_Ames assay 4 batches CONFIDENTIAL	Yes

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.2025.9537#support-information-section).