SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 24 May 2023

doi: 10.2903/j.efsa.2023.8065

Safety of apple fruit cell culture biomass as a novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Yolanda Sanz, Josef Rudolf Schlatter, Henk van Loveren, Wolfgang Gelbmann and Helle Katrine Knutsen

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 an apple fruit cell culture biomass as a novel food (NF) pursuant to Regulation (EU) 2015/2283 and intended as an ingredient for food supplements in adults. The cells have been sourced from the callus grown on a piece of apple placed on a solid medium under sterile conditions. The de-differentiated apple cells are then cultivated in liquid medium. The medium contains sucrose, vitamins, minerals, trace elements and the two synthetic plant hormone analogues, benzylaminopurine (< 0.1 mg/kg) and 2,4-dichlorophenoxyacetic acid (< 0.25 mg/kg). These plant hormones are regulated under the EU pesticide legislation and their residue levels in the NF are in compliance with the EU maximum residue levels. The main components of the NF are carbohydrates (including sugars and non-digestible carbohydrates), ash, proteins and smaller amounts of fatty acids and organic acids. Except for the amount of total fat and the organic acids (succinic and L-malic acid), the quantities of the compositional parameters of the NF and apple have little in common. The Panel considers that a provided subchronic toxicity study was not needed to establish the safety of this NF, when taking into account the source of the NF, i.e. apples, the production process, the low intended use level and the composition of the NF, despite the noted differences to apple. The Panel considers that the NF contains proteins, which were not detected in apple and which may be allergenic. The Panel concludes that the NF, an apple fruit cell culture biomass, is safe under the proposed conditions of use.

Keywords: novel foods, cell-culture derived food, apple cell culture biomass, food supplements

Requestor: European Commission

Question number: EFSA-Q-2020-00488

Correspondence: nif@efsa.europa.eu

Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Suggested citation: EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, Maciuk A, Mangelsdorf I, McArdle HJ, Naska A, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Cubadda F, Frenzel T, Heinonen M, Marchelli R, Neuhäuser-Berthold M, Poulsen M, Sanz Y, Schlatter JR, van Loveren H, Gelbmann W and Knutsen HK, 2023. Scientific Opinion on the safety of apple fruit cell culture biomass as a novel food pursuant to Regulation (EU) 2015/2283. EFSA Journal 2023;21(7):8065, 12 pp. https://doi.org/10.2903/j.efsa.2023.8065

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Table of contents

1. Introduction

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

On 14 April 2020, the company Mibelle Group Biochemistry submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to authorise placing on the Union market an apple fruit cell culture biomass as a novel food (NF).

The application requests to authorise the use of apple fruit cell culture biomass in food supplements.

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

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on apple fruit cell culture biomass as a NF.

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 (an) EFSA request(s) 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, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469.

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

3. Assessment

3.1. Introduction

In accordance to Article 3 of the Novel Food Regulation (EU) 2015/2283, the NF falls under category (vi), i.e. food consisting of, isolated from or produced from cell culture or tissue culture derived from animals, plants, microorganisms, fungi or algae.

It concerns the biomass from cultured cells derived from an edible Swiss apple variety (Uttwiler Spätlauber).

3.2. Identity of the NF

The NF is a biomass of cultivated and homogenised cells of the Swiss apple variety Uttwiler Spätlauber (*Malus domestica* Borkh.).

Malus domestica Borkh. (English common name, apple; synonym, *Malus pumila* auct.) is a member of the Rosaceae family and is cultivated throughout the world. The Uttwiler Spätlauber apple is an

[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.

edible cultivar originated from Switzerland. The main components of the NF are carbohydrates (which include sugars and also, ash and proteins).

3.3. Production process

The production process starts with cutting of the fruit and collecting specific sections of the apple, which are then placed on solid medium with the aim to induce the formation of a primary callus tissue comprised of dedifferentiated cells. The handling, cutting and transfer of the apple and the apple sections, respectively, to the solid medium is conducted under sterile conditions (biosafety cabinet class 2, sterilised equipment). Once a continuously growing callus culture has been established, some cells from the callus culture are used to form a stock culture and frozen backup cultures, which may be used to replace the stock culture every 5 years.

Cells from the stock culture are then transferred into sterile single-use 500 mL flasks with a medium of the same composition as the solid medium but without agar. The medium is mixed in house and contains sucrose, vitamins, minerals and trace elements. The medium contains also benzylaminopurine (BAP) (< 0.1 mg/kg) and 2,4-dichlorophenoxyacetic acid (2,4-D) (< 0.25 mg/kg), which are synthetic analogues of the plant hormones auxin and cytokinin, respectively. The cells are grown at room temperature with gentle shaking. Upon reaching certain values of specified parameters (pH, conductivity, packed cell volume, biomass, vitality index, refractive index, sugar content), about 10% of the content of a bottle is transferred under sterile conditions (biosafety cabinet class 2, sterilised equipment) to larger volumes of 1-L, then 20-L and finally, 50-L single-use, sterile biocontainers. The applicant provided information on the used biocontainers including the manufacturers and brand, specifications, method of sterilisation (gamma radiation in accordance with ISO 11137-2:2013). The material of the contact layer of the smaller biocontainers is linear low density polyethylene (LLDPE) and for the 20- and 50-L containers it is ultra-low-density polyethylene (ULDPE), both compliant to FDA 21CRF177.1520 according to the provided information. Upon complete metabolism of the sugars in the medium, the cell culture is harvested from the 50-L biocontainer by filtration and washed using 0.9% NaCl solution (Cas No 7647-14-5). The cultivation process in the liquid medium takes about 3–4 weeks.

Then the cell culture is mixed with 15% ethanol and homogenised under high pressure (1,000 bar) to disrupt the cells, followed by a temperature treatment at 113°C with a flow rate of 300 kg/h. Five batches of this product were freeze-dried and subjected to the compositional analyses as presented in Table 1. This product is considered to be the NF.

The product as intended to be marketed, however, is not intended to be stored as freeze-dried apple cell culture biomass, but instead of the freeze-drying step, the harvested, homogenised and sterilised biomass is spray-granulated onto an isomalt carrier and dried using a fluidised bed dryer. This product is a white, water-soluble mixture that contains 1.1–1.5% of the NF (biomass) and 98.5–98.9% isomalt (CAS No 64519–82-0), based on six batches produced in 2021. The source of isomalt is sugar beet *(Beta vulgaris)*. The product is packed and stored in closed polyethylene cans in the dark.

According to the information provided, the NF is produced in line with hazard analysis critical control points (HACCP) principles and ISO 22000 requirements. The applicant also indicates to produce in compliance with good manufacturing- and hygiene practices (GMP, GHP). Certificates of analysis have been provided for ingredients used for the medium and also ethanol, NaCl, the purified water and the isomalt.

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

Table 1 provides information on compositional parameters of five batches of the NF (i.e. the freeze-dried apple cell culture biomass before it is mixed with isomalt) analysed by an accredited laboratory.

Table 1: Compositional data of the freeze-dried NF without isomalt (g/100 g)

Parameter	Method	Batch no.				
		No. 1*	No. 2	No. 3	No. 4	No. 5
Moisture	Calculated	11.5	15.7	13.0	13.5	13.7
Dry residue	DIN 38409–1	88.5	84.3	87	86.5	86.3
Ash	DIN 38409–1	19.8	12.4	14	16.6	13.5
Sodium	ISO 11885	5.2	3.9	3.9	5.0	3.6
Total nitrogen	EN 25663	3.02	2.44	2.64	2.63	2.78
Protein (N × 6.25)	Calculated	18.9	15.2	16.5	16.4	17.4
Total fat	Soxhlet, gravimetric	2.4	0.6	1.2	1.1	1.0
Saturated fat	GC-FID	1.5	0.4	0.8	0.4	0.3
Succinic acid	Enzymatic	0.18	0.15	0.17	0.2	0.25
L-malic acid	Enzymatic	0.43	0.80	0.92	1.13	1.06
Saccharose	Enzymatic	2.8	4.9	4.2	3.8	4.1
D-Glucose	Calculated	4.0	6.7	5.4	5.2	5.4
D-Fructose	Enzymatic	11.4	19.2	15.3	14.8	15.3
Carbohydrate	Calculated	22.9	37.3	31.8	29.73	28.8
Non-digestible carbohydrates**	AOAC 985.29	23.9	17.8	22.4	21.3	22.9
Total polyphenols	Ph. Eur. 2.8.14	0.25	0.28	0.16	0.26	0.19
Tannins	Ph. Eur. 2.8.14	0.03	0.02	0.02	0.05	0.00

*: Batch No 1 was used for the *in vitro* genotoxicity tests.
**: Laboratory not accredited for this analysis.

Considering the source of the NF, i.e. apple, and because the dossier refers several times to the history of use of apples, EFSA requested the applicant to compare the composition of the apple cell culture biomass with the reference values provided in the OECD Consensus Document for Cultivars of Apple (OECD, 2019). This was provided and is summarised in Table 2.

Table 2: Composition of the freeze-dried NF (without isomalt) compared to apple (g/100 g) (OECD, 2019) per dry matter

Parameter	Apple cell culture biomass	Apple OECD (2019)	
	Mean*	Min.	Max.
Moisture	13.5	82.47**	86.20**
Ash	17.6 (20.4)	1.32	2.00
Sodium	5.0 (5.8)	0.004	0.0027
Protein	19.5 (22.6)	1.42	4.35
Total fat	1.4 (1.6)	1.18	3.62
Saturated fat	0.8 (0.93)	0.19	1.14
Succinic acid	0.2 (0.23)	***	
L-Malic acid	1.0 (1.2)	***	
Saccharose	4.6 (5.3)	14.35	20.5
D-Glucose	6.2 (7.2)	11.54	16.83
D-Fructose	17.6 (20.4)	32.63	48.55
Carbohydrates	34.9 (40.5)	94.7	95.74
Dietary fibre	25.0 (29)	8.52	16.62
Total polyphenols	0.3 (0.35)	0.523	2.724

*: Mean of the values of the five batches presented in Table 1; in brackets values are per dry matter.
**: Moisture per fresh weight; values of all other parameters for 'apple' are per dry matter.
***: OECD (2019) does not provide reference values, but refers to Fuleki (1995) who reported 0.08–0.97 mg/100 g for succinic acid and 0.56–2.17 g/100 g for L-malic acid; n.d. = no data.

EFSA notes the remarkable differences between the composition of the apple cell culture biomass and apple fruit, in particularly regarding the content of ash, sodium, protein and fibre. Except for the amount of 'total fat' and the organic acids (succinic and L-malic acid), the quantities of the compared compositional parameters of the NF and apple have little in common.

EFSA asked the applicant to provide information on the protein expression of the cultivated cells in comparison with the proteins found in the source, i.e. apple fruit of the variety of the Swiss variety Uttwiler Spätlauber. In addressing this request, the applicant provided a proteomic analysis. After cleavage of the proteins by tryptic digestion, the peptides were analysed by mass-spectrometry. The acquired mass-spectrometry data were processed for identification of the respective proteins using the search engine MSFragger (Fragpipe v17) and searched against the *Malus domestica* UniProt database entry (Proteome ID: UP000290289). The protein identification results were then imported in the software Scaffold and filtered for minimum of two peptides per protein, protein (False Discovery Rate) FDR value of 1% and peptide FDR values of 1%. According to this analysis, a total of 1,481 proteins were identified for the two samples. Whereas 1,400 proteins were identified in the biomass, 678 proteins were found in the apple fruit. Of the latter, 597 proteins were also found in the biomass, thus 803 of the 1400 proteins found in the biomass were not detected in the apple fruit.

According to the applicant's consideration, the reasons for this difference in the proteomic profile could be the physical characteristics of the sample materials, as the apple fruit has a firmer pulp compared to the cell culture biomass, which could affect the sample preparation process, but also the different environment in which the cells are growing and developing within an apple or within the cell culture. The applicant noted that the cells growing in the callus and later used to produce the stock or production culture undergo a significant dedifferentiation towards pluripotent apple cells and that involves that cells regain the ability of cell division. Therefore, a higher number of proteins can be expected to be expressed by these cells as compared to fully differentiated cells in a ripe apple.

In order to support this consideration and to further analyse the proteomic profile of the cultured apple cells, a gene ontology analysis was performed by the applicant with the 803 proteins that were only identified in the apple cell culture using the STRING database (https://string-db.org/). Ninety-four of these 803 proteins could be assigned to a certain function. Twenty-two of the proteins were considered to be especially involved in the metabolism of the dedifferentiated cells. Other proteins identified in the cultured apple cells, could be assigned to metabolic (n = 30) and biosynthetic processes (n = 12). The applicant noted that the currently available proteomic information on *Malus domestica* is limited, and therefore the gene ontology assignment is incomplete. The Panel conforms with this note.

While some of the differences between the composition of the biomass from cultured apple cells and apple may be explained by the dedifferentiation of the cultured cells that strive toward cell proliferation under atypical environmental conditions, with upregulation of protein translation combined with carbohydrate consumption as a source of energy, other differences, such as the high sodium content, was explained by the applicant to be probably a result of the NaCl solution used in the washing steps of the manufacturing process. The high fibre content was suggested by the applicant to be adaptive response to the shear forces during the cultivation process (when the cell cultures are gently rocked).

Information of potential chemical and microbiological contamination is only available for batches of the NF after it had been mixed with isomalt at an average ratio of about 1.4: 98.6 Table 3 presents the analyses of six such batches of the NF mixed with isomalt regarding heavy metals and microbiological contaminations.

Table 3: Contaminants of six batches of the NF mixed with isomalt

Parameter	Method	A	B	C	D	E	F
Heavy metals (mg/kg)							
Lead	EN ISO 15586	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0	≤ 1.0
Cadmium	EN ISO 15586	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Mercury	EN 1483	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1	≤ 0.1
Arsenic	EN ISO 11969	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Microbiological							
Total aerobic microbial CFU/g	EN ISO 21149	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10
Total yeast and mould CFU/g	EN ISO 16212	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10
Salmonella per 25 g	EN ISO 18415	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
E. coli per 25 g	EN ISO 18415	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S. aureus per 25 g	EN ISO 18415	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected; CFU: colony forming units.

According to the applicant, the first production batch of each year is screened for pesticide residues using GC/MS and LC/MS multiple residue screens and so far, the content of pesticides in all tested batches has been found to comply with Regulation (EC) No 396/2005[2].

In addition, the applicant reported the concentrations of the two plant hormone analogues used in the cultivation medium in five batches of the NF to be below levels of quantification. 6-Benzylaminopurine (6-benzyladenine) is a synthetic cytokinin derivative, which in combination with auxins induces cell dedifferentiation and proliferation. The EU maximum residue level (MRL) is 0.01 mg/kg according to Commission Regulation (EU) 2021/1841[3]. The other synthetic plant hormone analogue used in the production process is 2,4-D, which is an auxin plant hormone derivative (Endreb, 1994). It is also used in plant cell culture as a dedifferentiation hormone for callus induction. In the EU, the MRL for 2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D) is 0.05 mg/kg for apple fruit (Commission Regulation (EU) 2022/1363[4]). Both, BAP and 2,4-D, are regulated under the pesticide legislation.

Following an EFSA request, the applicant tested five batches of the NF (freeze dried before mixed with isomalt) for these two plant hormones (Table 4). 6-Benzylaminopurine was not detected (LOQ = 0.01 mg/kg). The concentrations of 2,4-D ranged between 0.052 mg/kg and 0.056 mg/kg. When taking into account the dry matter content of the freeze-dried NF, the Panel concludes that these concentrations are not of concern when considering that the EU MRL for 2,4-D of 0.05 mg/kg is applicable for fresh apples.

Table 4: Residual plant hormones analyses for five batched of the freeze-dried novel food (without isomalt)

Substance	Unit	Batch				
		A	B	C	D	E
BAP	mg/kg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
2,4-D*	mg/kg	0.052	0.054	0.052	0.053	0.056

*: Sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D.

The Panel notes that even though all the provided data presented in Table 3 regarding contaminants concern the mixture of the NF and isomalt and no analytical data, except for BAP and 2,4-D, on contaminants are available on the NF before it is mixed with isomalt, the Panel has no concerns regarding contamination when considering also the information provided on the production process and the intended uses.

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

[2] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32005R0396
[3] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32021R1841
[4] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32022R1363#ntr*1-L_2022205EN.01021101-E0001

3.4.1. Stability

The applicant performed stability tests with five batches of the NF mixed with isomalt, packed in 500 g polyethylene cans at a temperature of 25 °C and which have been stored and analysed after 8, 22, 32, 38 or 48 months, respectively. The parameters were light transmission of a 1% solution at 660 nm wavelength (according to USP 857), pH of a 1% solution in distilled water (according to USP 791), particle size distribution (according to USP 429) and loss on drying (according to USP 731).

Considering the production process and the compositional data, the Panel considers that the data provided sufficient information with respect to the stability of the NF at the intended use.

3.5. Specifications

Initially, the applicant had proposed specifications for a product with the apple cell culture biomass mixed with isomalt at a ratio between 1–10 (biomass) and 90–99 (isomalt), although according to the information provided on five batches the average (and range) ratio were 1.4 (1.1–1.5) biomass to 98.6 (98.5–98.9) isomalt.

EFSA noted to the applicant that the proposed specifications are unspecific and that even isomalt alone could comply with them and that the applicant should propose specifications which concern the biomass before it is mixed with isomalt. The applicant's response to this question is presented in Table 5.

Table 5: Specifications of the NF (i.e. apple cell culture biomass without isomalt) proposed by the applicant

Parameter	Unit	Specification
Moisture	g/100 g	10.9–15.5
Ash	g/100 g	11.8–20.8
Proteins	g/100 g	14.3–20.0
Fats	g/100 g	0.6–2.5
Carbohydrates	g/100 g	21.9–38.9
Non-digestible carbohydrates	g/100 g	17.1–25.2
Total sugars	g/100 g	17.1–32.6
Fructose	g/100 g	10.8–20.2
Glucose	g/100 g	3.8–7.0
Total phenols	g/100 g	0.15–0.29
Malic acid	g/100 g	0.41–1.19
Succinic acid	g/100 g	0.14–0.26

EFSA also asked the applicant whether there were any desired specific compositional or other characteristics present in the NF, which could be used as specification parameters. The applicant answered that there are no such compositional or other characteristics present in the NF.

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

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

3.6.1. History of use of the source

The applicant refers to the history of consumption of apple as the source of this NF.

3.6.2. History of use of the NF

In 2020, the NF (mixed with isomalt) has been assessed by the Advisory Committee on Novel Foods (ACNF) in Australia and New Zealand and was given the status of non-novel and non-traditional (FSANZ, 2022). According to this FSANZ record, no safety concerns were identified at an intended use level up to 10 mg/day of such mixture, which could contain 1–10% of the apple cell culture biomass and 90–99% of an isomalt carrier.

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

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

3.7.2. Proposed uses and use levels

According to the applicant, the maximum use level of the NF added to food supplements and when mixed with isomalt is 10 mg/day for the intended target population. The maximum concentration of the NF in this mixture is 1.5%.

When considering an average and range concentration of 1.4% and 1.1–1.5%, respectively, of the NF (i.e. apple cell culture biomass) in a mixture with isomalt, this would correspond to a daily intake up to 0.15 mg (or about 0.0021 mg/kg body weight (bw) considering an adult with 70 kg bw) of the apple cell culture biomass.

3.7.3. Precautions and restrictions of use

The applicant did not propose any precaution or restriction of use.

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

No ADME studies conducted with the NF were provided.

3.9. Nutritional information

The Panel considers, taking into account the composition of the NF (mainly carbohydrates, protein, ash and small amounts of fatty acids organic acids) and the proposed conditions of use with a maximum daily intake of 0.15 mg, that the consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

Originally, the applicant provided three genotoxicity tests (a bacterial reverse mutation test, an *in vitro* micronucleus test and an *in vitro* chromosomal aberration test) and a 90-day repeated dose oral toxicity study in rats with the mixture composed largely of isomalt and only 1.6% of the NF.

In response to an EFSA request to the applicant, noting that the sensitivity of genotoxicity studies is hampered when the test material is composed mainly of isomalt, the applicant conducted a new bacterial reverse mutation test and an *in vitro* micronucleus study performed with the NF (without isomalt) in compliance with Organisation for Economic Co-operation and Development (OECD) principles of GLP (OECD, 1998) and in the accordance with the test guidelines No. 471 and 487, respectively (OECD, 2016, 2020) briefly summarised in section 3.10.1.

3.10.1. Genotoxicity

In the *in vitro* bacterial reverse mutation assay four *Salmonella* Typhimurium strains (TA98, TA100, TA1535 and TA1537) and the *Escherichia coli* strain WP2 uvrA were treated with the NF (batch No No.1, Table 1) (Wisher, 2022). In this study the NF was non-mutagenic at concentrations up to 5,000 μg/plate, in the absence or presence of metabolic activation (S9). There was precipitation on the plates at the three highest concentrations (1,250, 1,500 and 5,000 μg/plate). The study author noted that no information had been provided on the purity of the reference item, *N*-ethyl-*N*′-nitro-nitrosoguanidine (ENNG), and as such no claim of compliance (with OECD 471) can be made for this information. However, the ENNG positive control used in this test induced the expected increase in revertant colony counts, which were within or close to the in-house historical control ranges for the relevant tester strains.

In an *in vitro* mammalian cell micronucleus test with human lymphocytes compliant with OECD Guidance No 487 and GLP with and without metabolic activation (Morris, 2022), the NF (batch No 1, Table 1) was not clastogenic or aneugenic up to the highest tested concentration of 5,000 μg/mL.

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 genotoxicity.

3.10.2. Subchronic toxicity

Over a period of 13 weeks, groups of 10 male and 10 female Sprague–Dawley rats received 0, 5, 10 or 16.7 mg/kg bw of a test item that contained the NF and isomalt at a ratio of 0.014:1, given by oral gavage with 10 mL sterile water/kg bw (Hsieh, 2016).

There were no reported test-item related adverse effects. The Panel notes however lack of compositional analyses of the NF used in this study and that some endpoints (i.e. functional observation battery, endocrine endpoints, local gastro-intestinal toxicity) were not investigated. Due to these limitations, the Panel did not consider this study further.

3.10.3. Human data

The Panel notes that there are no human studies available conducted with the NF.

3.11. Allergenicity

According to the compositional information provided by the applicant, the NF contains about 20% protein. Considering the proposed maximum intake of about 0.15 mg/day of the NF without isomalt, this would correspond to an intake of ~ 30 μg protein/day. According to the OECD (2019), apple allergy is one of the most common fruit allergies. The Panel notes the proteomic analysis performed by the applicant. According to this study, 803 proteins found in the NF could not be detected in an apple sample. The Panel considers that the NF contains proteins, which were not detected in apple and which may be allergenic.

4. Discussion

The applicant intends to market a biomass derived from cultured cells obtained from apples. The cells are sourced from the callus grown on a piece of apple placed on a solid medium under sterile conditions. The de-differentiated apple cells are then cultivated in liquid medium. The medium contains sucrose, vitamins, minerals and two synthetic plant hormone analogues, i.e. BAP (< 0.1 mg/kg) and 2.4-D (< 0.25 mg/kg). The information on the cultivation and the downstream process do not raise safety concerns. The NF is mainly composed of carbohydrates (including sugars and non-digestible carbohydrates), ash, proteins and smaller amounts of fatty acids and organic acids. EFSA notes the marked differences between the composition of the apple cell culture biomass and apple fruit, in particularly regarding the content of sodium, protein and fibre. Except for the amount of total fat and organic acids (succinic and L-malic acid), the quantities of the compared compositional parameters of the NF and apple have little in common. The NF is intended by the applicant to be used exclusively in food supplements for adults at a maximum daily intake of 0.15 mg. A subchronic toxicity study in rats was performed, but the report lacks compositional analyses of the NF and some endpoints. Due to these limitations, the Panel did not consider this study further. The Panel considers however that a subchronic toxicity study was not needed to establish the safety of this NF, when taking into account the source of the NF, i.e. apples, the production process, the low intended use level, and the composition of the NF, despite the noted differences to apple. The Panel considers that the NF contains proteins, which were not detected in apple and which may be allergenic.

5. Conclusions

The Panel concludes that the NF, an apple fruit cell culture biomass, is safe under the proposed conditions of use.

6. Steps taken by EFSA

1) On 01/07/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of apple fruit cell culture extract as a novel food.
2) On 03/12/2020, a valid application on on the safety of apple fruit cell culture extract, which was submitted by Mibelle Group Biochemistry, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1621) and the scientific evaluation procedure was initiated.
3) On 23/06/2021, 15/11/2022, and 09/05/2023 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.

4) On 22/09/2022, 13/04/2023, and on 19/05/2023, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 24/05/2023, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of Apple Fruit Cell Culture Biomass as a NF pursuant to Regulation (EU) 2015/2283.

References

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 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

Endreb R, 1994. Plant Cell Biotechnology. Springer, Germany. pp. 17, 18. ISBN 978–3–662-02996-1.

FSANZ (Food Standards Australia and New Zealand), 2022. Novel Food – Record of views formed in response to inquiries. Available online: https://www.foodstandards.gov.au/industry/novel/novelrecs/pages/default.aspx [Accessed: 1 November 2022].

Fuleki T, Pelayo E and Palabay RB, 1995. Carboxylic-acid composition of varietal juices produced from fresh and stored apples. Journal of Agricultural and Food Chemistry, 43, 598–607.

Hsieh, 2006. Unpublished study report on 90-Day repeated dose of oral toxicity in rats.

Morris, 2022. Unpublished study report on determination of the mutagenic potential of PhytoCellTec Md Nu with the Bacterial Reverse Mutation Test.

OECD (Organisation for Economic Co-operation and Development), 1998. OECD Principles of good laboratory practice (as revised in 1997). OECD series on principles of good laboratory practice and compliance monitoring, number 1, ENV/MC/CHEM(98)17, 41pp.

OECD (Organisation for Economic Co-operation and Development), 2016. Test No. 487: *In Vitro* Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264861-en

OECD (Organisation for Economic Co-operation and Development), 2019. Safety Assessment of Foods and Feeds Derived from Transgenic Crops, Volume 3: Common bean, Rice, Cowpea and Apple Compositional Considerations, Novel Food and Feed Safety, OECD Publishing, Paris. https://doi.org/10.1787/f04f3c98-en

OECD (Organisation for Economic Co-operation and Development), 2020), Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264071247-en

Wisher, 2022. Unpublished study report on apple cell culture extract powder: reverse mutation assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*.

Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
ADME	absorption, distribution, metabolism and excretion
BAP	benzylaminopurine
bw	body weight
CFU	colony forming unit
FAIM	food additive intake model
FDR	false discovery rate
GC/MS	gas chromatography/mass spectrometry
GHP	Good Hygiene Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
ISO	International Organization For Standardization
LC/MS	liquid chromatography/mass spectrometry
MRL	maximum residue level
NDA	Nutrition, Novel Foods and Food Allergens
NF	novel food
OECD	Organisation for Economic Cooperation and Development
USP	The United States Pharmacopeia