Safety of dried fruits of Synsepalum dulcificum as a novel food pursuant to Regulation (EU) 2015/2283.

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Safety of dried fruits of *Synsepalum dulcificum* as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on dried fruits of *Synsepalum dulcificum* as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is pitted and dried (by lyophilisation) fruits of *S. dulcificum*. The NF contains the glycoprotein miraculin (≤ 2.5%) which causes sour and acidic foods to taste sweet. The fruits have a documented history of consumption in Africa and products thereof can be found in different markets worldwide. Information on the production process and the composition of the NF is sufficient and does not raise safety concerns. The applicant proposes to use the NF as or in food supplements for the adult population, excluding pregnant and lactating women, at a maximum daily amount of 0.9 g. Taking into account these conditions of use, the Panel considers that the consumption of the NF is not nutritionally disadvantageous. The provided genotoxicity studies do not raise concerns for genotoxicity of the NF. The Panel concludes that the only dose tested in a 90-day oral toxicity study of 2,000 mg/kg body weight (bw) per day was not associated with adverse effects. By applying an uncertainty factor of 200, the Panel concludes that the NF is safe at an intake level of 10 mg/kg bw per day, corresponding to a maximum daily intake of 0.7 g of the NF for the target population, rather than 0.9 g/day as proposed by the applicant.

Keywords: Dried fruits of *Synsepalum dulcificum*, novel food, safety, food supplement

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

On 14 November 2018, the company Baïa Food Co. (Medicinal Gardens S.L.) submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place dried fruits of *Synsepalum dulcificum* on the Union market as a novel food.

The novel food (dried fruits of *S. dulcificum*) is intended to be used as or in food supplements. The target population is the adult population.

On 25 March 2019 and in accordance with Article 10(3) of Regulation (EU) 2015/2283, the Commission asked EFSA to provide a scientific opinion by carrying out the safety assessment for dried fruits of *S. dulcificum* as a NF in the context of Regulation (EU) 2015/2283.

2. Data and methodologies

2.1. Data

The safety assessment of this novel food (NF) is based on data supplied in the application, information submitted by the applicant following EFSA's requests for supplementary information and information provided by the EFSA Working Group on Compendium of Botanicals.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469[2].

A common and structured format 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 available (proprietary, confidential and published) scientific data, including both data in favour and not in favour to support the safety of the proposed NF.

This NF application includes a request for the protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data, requested by the applicant to be protected, comprise: toxicological information (studies on acute toxicity, genotoxicity and sub-chronic toxicity), human study and compositional studies.

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.

The information provided by the EFSA Working group on Compendium of botanicals is based on an extensive literature search on fruits of *S. dulcificum* using the following scientific databases: 'Scopus', 'Pubmed', 'Scifinder' and 'Web of Science'. This provided the basis for identifying scientific evidence available in peer-reviewed scientific papers in relation to substances contained in previously mentioned fruits of potential concerns, toxicological data and studies reporting adverse health outcomes in humans.

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

3. Assessment

3.1. Introduction

The NF, which is the subject of the application, is lyophilised pulp and skin of pitted fruits of *S. dulcificum* (Schumach. & Thonn) Daniell that belong to the family of Sapotaceae.[3]

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

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

[3] http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:789924-1

The NF falls under Regulation 2015/2283, Article 3(2)(a)(iv): food consisting of, isolated from or produced from plants or their parts.

The NF is proposed by the applicant to be used as or in food supplements. The target population is the adult population (except pregnant and lactating women).

3.2. Identity of the NF

The NF, named by the applicant 'Dried miracle berries, DMB' is pitted and dried (by lyophilisation) fruits of *S. dulcificum*.

The plant *S. dulcificum* is native to the forest regions of tropical West Africa and it can be found growing wild across the coast of the Gulf of Guinea (Juhé-Beaulaton, 2014). Fruits contain a glycoprotein called miraculin, responsible for a taste-modifying effect which causes sour and acidic foods to taste sweet (Kurihara and Beidler, 1968).

Miraculin is a protein consisting of 220 amino acids (Masuda et al., 1995) which belongs to the Kunitz-type soybean trypsin inhibitor (STI) family, but has lost its STI activity (Takai et al., 2013). It has a molecular weight of 24.6 kDa of which up to 13.9% (3.4 kDa) is represented by the sugar moiety, including glucosamine (31%), mannose (30%), fucose (22%), xylose (10%) and galactose (7%) (Theerasilp et al., 1989). It naturally occurs as a tetramer (98.4 kDa), a combination of four monomers grouped into two dimers bound by a disulfide bond.

Sanematsu et al. (2016) proposed a mechanistic model underlying the taste modifying effect of miraculin. It can bind to the amino-terminal domain of the human sweet taste receptor hTAS1R2 as an inactive form at neutral pH. Extracellular acidification protonates miraculin and the extracellular region of hTAS1R2, inducing partial activation of the sweet taste receptor. Membrane crossing of an unprotonated form of weak acids exerts further protonation of the intracellular domain of the sweet taste receptor leading to the full receptor activation.

3.3. Production process

The applicant provided a description of the production process, which follows Hazard Analysis Critical Control Points (HACCP) principles and general hygiene requirements. All materials in contact with the NF comply with applicable Union and National legislation on food contact materials.

The fruits are acquired from smallholder farms located in Ghana. They are manually harvested from mature cultivated plants, sorted, precooled and sent for the manufacturing process. Then, they are sorted once again, cleaned with demineralised tap water, and pitted mechanically. The remaining skins and pulps are then frozen and prepared for transport to the facility located in EU where they are dried by lyophilisation to preserve heat-sensitive compounds. The resulting dried cake is finally milled into a powder and stored.

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

3.4. Compositional data

The NF mainly consists of carbohydrates (up to 86.3%), and smaller amounts of proteins (up to 6%) and fats (up to 3.2%). According to the applicant, the key characteristic component of the NF is miraculin, a glycoprotein which can be found in amounts up to 2.5%.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information for five independent batches of the NF for proximate analyses and heavy metals (Table 1). Due to high contamination levels reported for some microbial parameters (data not shown), the production process was updated to the one described under Section 3.3 *Production process* in order to reduce the microbial content. Microbial parameters were analysed in seven new batches of the NF (Table 2).

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

Parameter (unit)	Batch no					Method/technique
	#1[a]	#2[a]	#3[a]	#4	#5	
Proximate analysis (g/100 g)						
Moisture	2.57	5.27	3.73	5.20	5.20	Gravimetric
Ash	3.70	3.53	4.23	5.00	5.40	Gravimetric
Total carbohydrate	86.3	81.0	77.7	79.4	80.6	Calculation
Sugars[b]	72.5	66.0	64.9	56.5	67.2	Ion chromatography/pulsed amperometry
Total protein	5.03	5.10	6.00	5.30	4.20	Kjeldahl[c]
Total fat	2.47	3.20	2.73	< 0.50	1.8	Gravimetric
Saturated fatty acids	1.1	1.33	0.70	< 0.10	0.80	GC-FID
Polyunsaturated fatty acids	0.7	1.23	1.2	< 0.10	0.6	GC-FID
Monounsaturated fatty acids	0.63	0.63	0.8	< 0.10	0.4	GC-FID
Sodium chloride	0.029	0.021	0.036	0.043	0.037	ICP-MS spectroscopy
Fibre	-	1.9	5.6	5.1	2.8	Gravimetric
Energy (kJ/100 g)	1,619	1,597	1,569	1,481	1,531	Calculation
Energy (kcal/100 g)	387	377	371	349	361	Calculation
Heavy metals						
Arsenic (mg/kg)	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	ICP-MS
Cadmium (mg/kg)	0.069	0.063	0.14	0.088	0.070	
Mercury (mg/kg)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	
Lead (mg/kg)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	

GC-FID: gas chromatography with flame-ionisation detection; ICP-MS: inductively coupled plasma mass spectrometry.
(a): Analyses done in triplicates.
(b): Total of glucose, fructose, saccharose, maltose and lactose.
(c): Total nitrogen × 6.25.

Table 2: Microbiological quality of the NF

Parameter (unit)	Batch no							Method
	#9	#10	#11	#12	#13	#14	#15	
Microbial parameters (CFU/g)								
Total aerobic plate count	1,100	4,600	6,900	380	5,900	45[b]	82[b]	Internal method based on UNE EN ISO 4833-1
Presumptive *Bacillus cereus*	< 40	< 40	< 10	< 10	< 10	< 10	< 10	Colony count/BKR 23/06-02/10/ UNE EN-ISO 7932:2005
Sulfite-reducing *Clostridia*	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Internal method based on ISO 15213
Enterobacteriaceae	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Internal method based on UNE EN ISO 21528-2
Yeasts and moulds	370	< 10	100	40[b]	150	60[b]	100	Internal method based on ISO 21527-1 and ISO 21527-2
Staphylococci coagulase +	< 10	< 10[a]	< 10	< 10	< 10	< 10	< 10	Internal method based on ISO 6888-2
Escherichia coli β glucuronidase +	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Internal method based on chromidID™ Coli Agar (COLI ID-F)
Faecal coliforms	< 10	< 10	< 10	< 10	< 10	< 10	< 10	Internal method based on chromidID™ Coli Agar (COLI ID-F)

CFU: colony forming units.
(a): Reported as *Staphylococcus aureus* count.
(b): Estimated count.

The applicant also provided mineral contents measured by inductively coupled plasma/mass spectrometry or optical emission spectroscopy (ICP-MS or ICP/OES) and fatty acid profiles measured by gas chromatography with flame-ionisation detection (GC-FID) of the same first five independent batches of the NF (data not shown).

Furthermore, the applicant performed a determination of the total polyphenolic content of three different batches of the NF, using spectrophotometry (Folin–Ciocalteu) (Table 3).

Table 3: Total polyphenolic content in the NF

Parameter (unit)	Batch no[(a)]			Analytical technique
	#1	#2	#6	
Total polyphenols (mg GAE/g)	4.25	4.54	4.20	Spectrophotometry

GAE: Gallic acid equivalents.
(a): Analyses done in triplicates.

The applicant used a validated in-house method based on proton nuclear magnetic resonance (^{1}H-NMR) analysis (which captures part of the glycosylation pattern of miraculin) to quantify miraculin in five different batches of the NF. The results were presented as the percentage of miraculin present in the NF (w/w) (Table 4).

Table 4: Content of miraculin in the NF

Parameter (unit)	Batch no					Analytical technique
	#1	#2	#5	#6	#7	
% of miraculin in the NF (w/w)	1.7	1.7	2.1	1.8	2.0	^{1}H-NMR

^{1}H-NMR: proton nuclear magnetic resonance.

The results showed the range of miraculin between 1.7% and 2.1% of the total weight of the NF.

Additionally, the applicant provided concentrations of polycyclic aromatic hydrocarbons (PAHs) in four independent batches of the NF, measured by gas chromatography–mass spectrometry (GC–MS) which were below maximum levels for PAHs in food supplements.[4]

Concentrations of chlorate were quantified in batches of the NF in amounts up to 0.046 mg/kg but are not above the maximum residue level for berries and small fruits (0.05 mg/kg).[5]

Regarding concentrations of mycotoxins, the applicant provided certificates of analyses by liquid chromatography with tandem mass spectrometry (LC–MS/MS) for five independent batches of the NF and no results (data not shown) exceeded maximum levels in regulated foods (Regulation (EC) No 1881/2006).

EFSA retrieved a study by Njoku et al. (2016), which reported the occurrence of alkaloids and of some antinutrients in the pulp of *S. dulcificum* fruits. Upon a request to comment on these findings, the applicant provided an analytical report on the content of oxalates, trypsin inhibitor and pyrrolizidine alkaloids in three different batches of the NF (Table 5). The applicant also submitted a study by Menéndez-Rey et al. (2021) in which the concentration of lectins in three batches of the NF was quantified by haemagglutination assays in human blood.

Table 5: Content of oxalates, trypsin inhibitor and pyrrolizidine alkaloids in the NF

Parameter (unit)	Batch no			Analytical technique
	#5	#7	#8	
Oxalic acid (g/100 g)	0.06[(a)] 0.09[(b)]	0.07[(a)] 0.10[(b)]	0.05[(a)] 0.07[(b)]	HPLC
Trypsin inhibitor (TIU/mg dw)	0.80	0.81	0.97	UV-VIS

[4] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5.
[5] Commission Regulation (EC) No 2020/749 of 4 June 2020 amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for chlorate in or on certain products. OJ L 178/7, 8.6.2020, p. 14.

Parameter (unit)	Batch no			Analytical technique
	#5	#7	#8	
Sum of pyrrolyzidine alkaloids (LB, μg/kg)[(c),(d)]	7.2	Not detected	Not analysed	LC-MS/MS

TIU: trypsin inhibitor unit; dw: dry weight; LB: lower bound; HPLC: high-performance liquid chromatography; UV–VIS: ultraviolet–visible; LC–MS/MS: liquid chromatography–tandem mass spectrometry; LOQ: limit of quantification.
(a): Expressed as oxalic acid.
(b): Expressed as calcium oxalate.
(c): Also quantified in the batch #1 in amount of 5.6 μg/kg.
(d): Analyses of 21 pyrrolizidine alkaloids were performed (LOQ range 0.5–2 μg/kg).

According to the reported results, the amounts of oxalic acid in the NF ranged between 0.05 and 0.07 g/100 g (i.e. 500 and 700 mg/kg). EFSA (2018) noted the oxalate content in fonio (*D. exilis*) and wheat bran of 2,600 and 4,600 mg/kg, respectively. Trypsin inhibitor content in the NF varied between batches (0.80–0.97 TIU/mg dw), but was lower than those reported in chia seeds (13 TIU/mg dw) by De Souza et al. (2017), and in soybean (43-84 TIU/mg) and common bean (21–28 TIU/mg) by Guillamon et al. (2008). The sum of pyrrolizidine alkaloids in the NF was quantified in amounts up to 7.2 μg/kg, which is below the future maximum level for food supplements of 400 μg/kg.[6] Haemagglutinating activity of the NF was low and comparable with the activity of blueberries and raspberries (Menéndez-Rey et al., 2021).

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

3.5. Stability

In order to demonstrate the stability of the NF, the applicant conducted a study of shelf-life under normal storage conditions (20 ± 2°C, relative humidity 50 ± 10%) over a 24-month period with one batch of the NF (#1). Sensory parameters (smell, colour, flavour and texture) were not changed throughout the whole testing period (data not shown), while changes in moisture, water activity and pH were similar (Table 6). Furthermore, microbial parameters were measured in the 6th, 12th and 24th month of the study (Table 7).

Table 6: Physicochemical stability of batch #1 of the NF

Parameter (unit)	t (months)				Method
	0	7	12	24	
Moisture (g/100 g)	1.8	1.1	2.4	2.7	Thermogravimetry
Water activity	0.11	0.11	0.13	0.12	Hygrometric
pH	3.2	3.7	3.2	3.3	Potentiometric

Table 7: Microbial stability of batch #1 of the NF

Microbial parameters (CFU/g)	t (months)			Method
	6	12	24	
Moulds	55[(a)]	< 10	91[(a)]	Plate count/ISO 21527-2 2008
Yeasts	< 400	< 10	< 10	Plate count/21527-2 2008
Escherichia coli β-glucuronidase+ count	< 10	< 10	< 10	Plate count/ISO 16649-2:02
Positive coagulase staphylococci count	< 10	< 10	< 10	Plate count/ISO 6888-1:00
Presumptive *Bacillus cereus*	40	< 10	400	Plate count/ISO 7932-2:05
Listeria monocytogenes (in 25 g)	Absence	Absence	Absence	Real time PCR/Internal method
Salmonella spp (in 25 g)	Absence	Absence	Absence	Real time PCR/Internal method

CFU: colony forming units; PCR: polymerase chain reaction.
(a): Estimated value.

[6] Commission Regulation (EU) No 2020/2040 of 11 December 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of pyrrolizidine alkaloids in certain foodstuffs. OJ L 420/1, 14.12.2020, p. 1–4.

In order to further support the stability of the NF, the applicant conducted additional analyses on four batches of the NF, each batch tested only at one time point (Table 8). The applicant also submitted results of sensory analyses for those four batches of the NF which reported no changes (data not shown).

Table 8: Stability of four different batches of the NF

Parameter (unit)	Batch No				Method
	#7	#2	#1	#6	
t (months)	**18**	**26**	**30**	**36**	
Microbial parameters (CFU/g)					
Aerobic microorganism count	4,100	11,000	3,500	3,700	Internal method based on EN ISO 4833-1
Moulds and yeasts count	< 40	< 10	< 10	< 10	Internal method based on ISO 21527-2
Enterobacteriaceae count	< 10	< 10	< 10	< 10	Internal method based on EN ISO 21528-2
Total coliform count	< 10	< 10	< 10	< 10	Internal method based on chromID™ Coli Agar (COLI ID-F)
Escherichia coli β-glucuronidase+ count	< 10	< 10	< 10	< 10	Internal method based on chromID™ Coli Agar (COLI ID-F)
Positive coagulase staphylococci count	< 10	< 10	< 10	< 10	Internal method based on ISO 6888-2
Physico-chemical parameters					
Water activity	0.125	0.129	0.138	–	
pH	3.1	3.0	3.3	–	

The applicant also conducted a stability test of the NF to temperature and pH. The aim of the study was to determine whether varying temperature or pH conditions will denature and consequently inactivate miraculin. The volunteers tested taste-modifying properties of the NF after the NF was exposed to different temperatures (at –80, –20, 4, 20, 37 and 60°C) for 1 h or 10 min (n = 10). The results indicated a loss of taste-modifying properties of the NF at 60°C, which could not be restored anymore. A pH stability assay suggested that miraculin is stable and functional at pH 2–10.

The Panel notes that stability data in Tables 7 and 8 refer to the batches of NF produced with the initial production process, and thus not all microbial parameters at every time point measured were consistent or in line with the specifications of the NF. However, considering physico-chemical characteristics of the NF, as well as the fact that the applicant produced new batches of the NF of acceptable microbiological quality (Table 2), the Panel considers the provided stability data as sufficient.

3.6. Specifications

The specifications of the NF are indicated in Table 9.

Table 9: Specifications of the NF

Description: The NF is lyophilised pulp and skin of pitted fruits of *Synsepalum dulcificum* (Schumach. & Thonn) Daniell	
Parameter (Unit)	**Specification**
Moisture (g/100 g)	< 6
Ash (g/100 g)	3.5–8.5
Total carbohydrates (g/100 g)	70–87
Sugars (g/100 g)	50–75
Fibre (g/100 g)	1–6.5
Total protein (g/100 g)	3.5–6.0
Total fat (g/100 g)	> 0.50–3.50
Saturated fatty acids (g/100 g)	> 0.20–1.40
Sodium chloride (g/100 g)	0.015–0.045

Description: The NF is lyophilised pulp and skin of pitted fruits of *Synsepalum dulcificum* (Schumach. & Thonn) Daniell	
Parameter (Unit)	**Specification**
Energy (kJ/100 g)	1,180–1,620
Energy (kcal/100 g)	282–387
Total polyphenols (mg/g)	4–5
Miraculin (%)	1.5–2.5
Total aerobic count	< 10,000
Bacillus cereus (presumptive)	< 100
Sulfite-reducing *Clostridia*	≤ 30
Total Enterobacteriaceae	< 100
Moulds and yeasts	< 500

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

3.7.1. History of use of the source

There is a documented history of consumption of fruits of *S. dulcificum*, which were consumed by natives of Western and Central Africa, since the 18th century (Labat, 1730; Dalzel, 1967; Juhé-Beaulaton, 2014). The fruits were mainly consumed prior to eating meals for altering their taste.

According to the applicant, nowadays *S. dulcificum* is mainly cultivated in Asia (Taiwan and China), the USA and Latin America (Ecuador, Colombia and Puerto Rico), with cultivation declining in its native African regions (the Gulf of Guinea). The fruits and products thereof are available in different forms (as fresh and dried fruits, juices, chewing tablets, freeze-dried powders, canned fruits, tablets etc.) and are sold mainly via online stores (websites). In the USA, some bars, restaurants and hotels have these fruits on their menus. According to the applicant, miraculin does not have a GRAS status given by the FDA since it is considered to be a food additive and no application has been submitted for its authorisation. Fruits can be cultivated and sold while falling under the remit of the USDA. Furthermore, the applicant has consulted with the US FDA adverse event reporting system (FAERS) public dashboard and did not find any entry related to miraculin or the raw material (fruits). In Japan, the fruits are considered as food and can be sold without restrictions, as stated by the applicant.

3.8. Uses and use levels and anticipated intake

3.8.1. Target population

The target population proposed by the applicant is the general adult population with the exception of pregnant and lactating women. The applicant proposed to exclude those subgroups of the population since there is insufficient information available for their safety assessment.

3.8.2. Proposed uses and use levels

The applicant proposed to use the NF as or in food supplements (in various forms such as capsules, powders, orally dissolving tablets, lozenges, liquids, chewing gums and gels) at a maximum dose of 0.9 g per day (divided over three servings).

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

The applicant conducted an *in vitro* study to analyse the digestibility of a protein extract of the NF (miraculin) through incubation with different enzymes (Menéndez-Rey, 2018). The non-proteolytic enzymes (DNAse, RNAse, α-amylase, lactase) and proteolytic enzymes (pepsin, peptidase D, carboxypeptidase M, trypsin) were used to assess whether they can degrade miraculin under optimal (non-physiological) conditions. Miraculin remained stable after enzymatic treatment with non-proteolytic and some proteolytic enzymes (peptidase D and carboxypeptidase M) but was degraded after the treatment with pepsin and trypsin, which is in line with natural protein digestion in the GI tract, as stated by the authors.

3.10. Nutritional information

As previously mentioned in Section 3.4 *Compositional data,* relevant information on nutrient and antinutrient composition of the NF is available. Taking into account the maximum daily dose of the NF proposed by the applicant (i.e. 0.9 g/day), the Panel considers that the consumption of the NF is not nutritionally disadvantageous.

3.11. Toxicological information

The applicant provided several toxicological studies on the NF. These studies are listed in Table 10.

Table 10: Summary of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Study No. IF-74616 (CERETOX, 2018a)	Bacterial reverse mutation test (GLP, OECD TG 471)	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	Up to 5,000 μg/plate (absence and presence of S9 mix)
Study No. 20229053 (Charles River, 2020a)	Bacterial reverse mutation test (GLP, OECD TG 471)	*S.* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	Up to 5,000 μg/plate (absence and presence of S9 mix)
Study No. IF-74516 (CERETOX, 2018b)	*In vivo* mammalian erythrocyte micronucleus test (GLP, OECD TG 474)	Wistar rats	2,000 mg/kg bw/day (oral gavage)
Study code: 20/020-013C (Charles River, 2020b)	*In vitro* mammalian cell micronucleus test (GLP, OECD TG 487)	Mouse lymphoma L5178Y TK+/– 3.7.2C cells	Up to 1,000 μg/mL
Study No. IF-81517 (CERETOX, 2018c)	Acute oral toxicity study by Up-and-Down Procedure (GLP, OECD TG 425)	Wistar rats	5,000 mg/kg bw per day
Study No. 73416 (CERETOX, 2018d)	90-day repeated dose oral toxicity study with a 14-day recovery period (GLP, OECD TG 408, limit test)	Wistar rats	2,000 mg/kg bw per day

bw: body weight; CERETOX: Centre de Recerca en Toxicologia; GLP: Good Laboratory Practice; OECD Organisation for Economic Co-operation and Development

3.11.1. Genotoxicity

A bacterial reverse mutation test (CERETOX, 2018a) was performed according to OECD Test Guideline No 471 (OECD, 1997) and in compliance with the principles of Good Laboratory Practice (GLP). Using *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537 and the plate preincubation method, the NF was tested in the main and confirmatory tests at dose levels up to 5.0 mg/plate (all strains), both in the absence and presence of a metabolic activation system, MAS (S9-mix). The Panel notes that no certificate of analysis for MAS has been provided. As a negative control (NC), sterile water was used and as positive controls (PC), sodium azide (without S9) and 2-aminoanthracene (2-AA) (with S9). According to OECD TG No. 471, 2-AA should not be used as the sole indicator of the efficacy of the S9-mix. The Panel notes that in the main and confirmatory tests, for strain TA102 (both with and without S9) the number of revertant colonies in NC was out of the range of historical control data (reaching almost 2-fold increase). Some colony counts in the confirmatory test were discarded, due to contamination. Furthermore, an increase of revertant colonies in the confirmatory test for strain TA1535, while not dose-dependent, reached significant increases of almost 2.5-fold in mid-dose groups in comparison to NC. However, in the main test, no significant increase was observed.

The Panel considered that an additional bacterial reverse mutation test, using a preincubation method with all strains or a mammalian system (OECD TG 476; OECD, 2016a) was needed for better assessment of the mutagenic potential of the NF.

Upon request, the applicant submitted an additional bacterial reverse mutation test (Charles River, 2020a). The study was performed in accordance with OECD TG No. 471 (OECD, 1997), EC Guideline No 440/2008[7] and GLP. *S. typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to the NF in two independent experiments, using a direct plate assay and a pre-incubation assay, both in the absence and presence of a MAS (S9-mix). Based on the dose-range finding study, the NF was tested up to 5 mg/plate. The NF did not induce a significant dose-related increase in the number of revertant colonies in any of the five tester strains, both in the absence and presence of the S9-mix.

An *in vivo* assessment of genotoxicity was also undertaken, using the rat erythrocyte (sampled from peripheral blood) micronucleus assay (CERETOX, 2018b). This test was conducted in accordance with OECD TG 474 (OECD, 2016b) and in compliance with GLP. The NF was administered twice (24 h apart) via oral gavage to 5 male Wistar rats in a dose of 2,000 mg/kg body weight (bw). Blood samples from the tail vein were obtained ~ 43 h after the treatment. At least 2,000 erythrocytes per animal were analysed, counting the number of polychromatic erythrocytes (PCE) and at least 4,000 erythrocytes for the proportion of micronuclei (MN)/PCE. No statistically significant increases in the number of cells with micronuclei were observed compared to the negative control (0.5% carboxymethylcellulose sodium salt, CMC).

The Panel considers that it is not known whether and to what extent the NF or its metabolites have reached the target cells. Thus, this study is not sufficient to properly address the genotoxic potential of this NF and the applicant was requested to perform an *in vitro* mammalian cell micronucleus assay (OECD TG 487; OECD, 2016c). This testing strategy is in line with the EFSA Scientific Committee recommendations of *in vitro* tests to be used as the first step in testing (EFSA Scientific Committee, 2011).

As requested, the applicant provided an *in vitro* mammalian cell micronucleus test (Charles River, 2020b), conducted in accordance to OECD TG 487 (OECD, 2016c) and in compliance with the principles of GLP. Mouse lymphoma L5178Y $TK^{+/-}$ 3.7.2C cells were used as a test system. A 3-h treatment with S9-mix and 3-h and 24-h treatments without S9-mix were performed. Sampling was performed 24 h after the beginning of the treatment. The examined concentrations of the NF were 31.25, 62.5, 125, 250, 500 and 1,000 μg/mL. Since the positive control did not produce expected results in the short-term treatment with S9-mix (due to technical issues as presumed by the study authors), this test was repeated. The insolubility of the NF was observed in the final treatment medium at the end of the treatment at 500 and 1,000 μg/mL. No cytotoxicity was observed after any treatment. None of the treatment concentrations caused a biologically or statistically significant increase in the number of micronucleated cells when compared to the negative control values (vehicle; 0.5% CMC).

The Panel concludes that the provided studies do not raise concerns for the genotoxicity of the NF.

3.11.2. Acute toxicity

In an acute oral toxicity study in rats (CERETOX, 2018c), 3 female Wistar rats were administered the NF up to 5,000 mg/kg bw in a study design following an Up-and-Down procedure (UPD). The study was conducted in accordance with OECD TG 425 (OECD, 2008) and no adverse effects were recorded. The Panel considers that in general, acute toxicity studies are not pertinent for the safety assessment of NFs.

3.11.3. Subchronic toxicity

The applicant submitted results of a 90-day repeated dose toxicity study in male and female Wistar rats (CERETOX, 2018d), conducted in accordance with OECD TG 408 (limit test; OECD, 1998) and in compliance with GLP. Rats (10/sex per group) were randomised in the negative control group (0.5% CMC) and one dose group receiving the NF by oral gavage at a dose level of 2,000 mg/kg bw per day. Additional groups (5/sex per group) receiving 0 (control) or 2,000 mg/kg bw per day were included in order to assess the reversibility or progression of any test item-related changes after a 14-day recovery period. No systemic clinical signs, behavioural changes or mortality were observed at either control or treated group, as well as no effects on food and water intake. Furthermore, no test item-related effects in the treatment group were observed on bw and bw gain in comparison to the control.

[7] COUNCIL REGULATION (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Part B: Methods for the Determination of Toxicity and other health effects. Guideline B.13/14: "Mutagenicity: Reverse Mutation Test using Bacteria". OJ No. L 142, 31.05.2008. p. 248–256.

Some haematological, coagulation and clinical blood chemistry parameters, as well as organ weights, reached statistical significance, but did not show consistency between main and recovery groups, sex or trend over time or were of small magnitude and thus were considered as incidental (Annex A). The only relevant findings recorded at the histological examination were at the lung level. High incidence of foci of alveolar macrophages or foreign body granulomas was noted in control or treated rats, respectively. This is considered related to the experimental procedures (i.e. gavage administration) and to the characteristics of the test item that is a suspension at acidic pH.

Even though only one dose level is used in a limit test, the Panel considers that this study with 2,000 mg/kg bw does not raise concern in regard to sub-chronic toxicity of the NF.

3.11.4. Human data

The applicant submitted one sensory study with healthy young adults (Aguiló Aguayo and Echeverria Cortada, 2018) conducted with the NF and referred to several publications investigating the efficacy and sensory properties of *S. dulcificum* fruits in human subjects (Table 11).

In a sensory test (Aguiló Aguayo and Echeverria Cortada, 2018), nine healthy volunteers were recruited to evaluate the effect of different miraculin concentrations (up to 300 mg) over the time period of 60 min on the sweetness and sourness perception, using visual analogue scales. A solution consisting of sugars and acids was tasted after the NF was applied on the subject's tongue in form of chewable tablets and was left to dissolve for 2 min. The sweetness perception reached its peak in the first 5 min and was highly reduced at the end of the test period. The duration of the taste-altering effect (up to 120 mins) was in line with what has been reported elsewhere (Kurihara and Beidler, 1968; Igarashi et al., 2013; Rodrigues et al., 2016; Tafazoli et al., 2019). According to the applicant, none of the participants reported any adverse effects.

Other studies involving human subjects assessing the taste altering properties of different products derived from *S. dulcificum* fruits are summarised in Table 11.

Table 11: Overview table of human studies

Reference	Study Design	Study Population	Duration of Study	Doses; route of administration
Wong and Kern (2011)	Randomised, blinded cross-over	13 females	4 non-consecutive trial days within 2 weeks	Not available (freeze dried pill form); Supralingual
Igarashi et al. (2013)	Sensory evaluation experiment	18 subjects (8 males and 10 females, around 20 years of age)	Single occasion	Not available (thawed freeze-dried miracle fruit); Buccal
Rodrigues et al. (2016)	Time-intensity test	12 healthy adults	Single occasion (triplicates)	300 mg of Frooties®; Supralingual
Capitanio et al. (2011)	Sensory evaluation experiment	10 healthy adults (3 males and 7 females)	Three occasions	25 mg of miraculin; Supralingual
Hudson et al. (2018)	Sensory evaluation experiment	97 (1st experiment), 84 (2nd experiment) and 80 (3rd experiment) subjects	2 non-consecutive days	1 tablet (stated to be an equivalent of 1 miracle fruit berry); Buccal
Andrade et al. (2019)	Time-intensity test	11 adults (8 women and 3 men)	2 non-consecutive occasions	150, 300 and 600 mg of spray-dried miracle fruit; Supralingual
Wilken and Satiroff (2012)	Non-randomised control trial	8 participants on chemotherapy (4 in the treatment group and 4 in placebo)	2 weeks	6 Miracle Fruits™ per day.
Tafazoli et al. (2019)	Sensory study	6 panellists	Single occasion	0.08 g of miracle fruit powder; Supralingual

The Panel notes that none of the available human studies investigated safety-related parameters of the NF or products of its source, but rather its taste-altering properties. While considering this and

other inherent limitations of these studies for their use in a safety assessment, the Panel notes that the authors did not report adverse effects regarding possible local effects (e.g. irritation of the tongue and/or mouth) at a maximum reported dose of 600 mg (Andrade et al., 2019) or duration of 2 weeks (Wilken and Satiroff, 2012).

Upon a request to provide more evidence on the safety of the NF in the context of chronic consumption in humans and possible prolonged or permanent taste alteration, the applicant submitted an expert opinion. The expert opinion concluded that data available from sensory studies suggest that the taste-altering effect of miraculin has a rapid onset and disappearance with no desensitising impact on the receptors.

The Panel notes a lack of data addressing to possible change in taste preference after long-term use.

3.12. Allergenicity

The total protein content of the NF is approx. 5-6% (w/w) and the glycoprotein miraculin represents about 15-40% of this amount. Glycoproteins are often allergenic (Woodfolk et al., 2015; Lei and Grammer, 2019). According to the applicant, no allergic reactions upon the consumption of fruits of *S. dulcificum* have been reported. Some species belonging to Sapotacea family are also known by their latex production (De Faria et al., 2017) and cross-reactivity between latex and fruit allergens (latex-fruit syndrome) has been described (Brehler et al., 1997).

Sequence homology has been carried out (Cózar, 2018) between the miraculin amino acid sequence (Uniprot. Id: P13087) and all the protein amino acid sequences of the National Center for Biotechnology Information (NCBI) database using protein BLAST analysis tool (Basic Local Alignment Search Tool) (Altschul et al., 1990). The results revealed that the sequences with higher homology to miraculin (> 50% sequence identity) were proteins from peach, sesame and bitter lemon (Kunitz trypsin inhibitor 2) and from tomato (miraculin precursor). In the same study, the sequential identity between miraculin and widely known protein allergens from other plants was assessed. At a cut off higher than 50%, sequence identities were detected with allergens from latex (83%), peach (50%) soy (53%) and peanut (80%) but with low query cover %. The author concludes that "no significant homology between miraculin and widely known pan-allergens has been found using BLAST". The Panel considers these results as preliminary and they do not allow to draw definitive conclusions on cross-reactivity.

A similar *in silico* evaluation using the allergen database 'Allergen Online'[8] was performed to evaluate the potential allergenicity of miraculin (Tafazoli et al., 2019, 2020). An identity match higher than 35% over an 80 amino acid window was taken as a significant sequence homology and evidence for cross-reactivity. Significant alignments (≥ 35% identity) with seven proteins, including the Kunitz trypsin inhibitor 2 from soy and proteinase inhibitor and aspartic protease inhibitor from potato, were found. In the same study, miraculin was shown to be rapidly and completely digested under simulated physiological gastric conditions (pepsin).

The applicant also performed an internal preliminary enzyme-linked immunosorbent assay (ELISA) screening with the major food allergens. The results showed that the NF tested positive for peanut allergens.

By combining all these results, the Panel considers that cross-reactivity exists between the NF and peanut allergens as well as a potential cross-reactivity with latex, peach and soy allergens.

4. Discussion

The NF is pitted and lyophilised berries of *S. dulcificum* Daniell and contains glycoprotein miraculin (≤ 2.5%). The applicant proposes to use the NF as or in food supplements in various forms which will allow the delivery of miraculin into the mouth, for the general adult population (with the exception of pregnant and lactating women). The maximum daily amount of the NF to be consumed is 0.9 g, as proposed by the applicant.

The Panel considers that, taking into account the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous and the exposure to the contaminants present in the NF is not of toxicological relevance.

The only dose tested in the 90-day oral toxicity study of 2,000 mg/kg bw per day was not associated with adverse effects. As the proposed daily intake of the NF of 0.9 g/day, corresponding to 12.9 mg/kg

[8] http://www.allergenonline.org

bw per day for an adult with default bw of 70 kg (EFSA Scientific Committee, 2011), the resulting margin of exposure (MOE) is 155. The Panel considers that the MOE of 200 (uncertainty factor 10 × 10 for inter- and intraspecies extrapolation and additional uncertainty factor 2 to account for lack of chronic toxicity data) is necessary to ensure the safety of the NF. Thus, the Panel derives a maximum intake level of 10 mg/kg bw per day for the NF, which corresponds to 0.7 g/day for an adult of 70 kg bw.

Regarding the specific proposed use of this NF (supralingual), the Panel considers that there was no irritation in the non-glandular (fore-stomach) epithelium from the submitted *in vivo* subchronic oral toxicity study, which would lead to concern regarding local effects in human tongue and mouth. Furthermore, no such effect was reported in the available sensory studies with human subjects or in the history of use of the source of the NF, as well as in the limited post-market surveillance data regarding the products similar to the NF (i.e. containing miraculin).

No studies reporting or investigating possible prolonged alterations in taste sensation following chronic consumption of the NF or of *S. dulcificum* fruits were identified. However, the history of consumption of miraculin and *S. dulcificum* fruits do not reveal an indication for permanent taste-alterations. Despite the uncertainties, the Panel is of the view that the likelihood of prolonged or permanent taste alterations is low, considering that the taste cells have regenerative capabilities with an approximate life span of 8–22 days (Beidler and Smallman, 1965; Perea-Martinez et al., 2013) and that the miraculin taste-altering effect is reversible and typically lasts between 30 and 120 min (Kurihara and Beidler, 1968; Igarashi et al., 2013; Rodrigues et al., 2016; Aguiló Aguayo and Echeverria Cortada, 2018).

5. Conclusion

The Panel concludes that the NF is safe for use as or in food supplements at the maximum intake level of 0.7 g/day for the target population, i.e. adults excluding pregnant and lactating women.

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, i.e. compositional studies, and toxicological information (genotoxicity and sub-chronic toxicity studies).

6. Steps taken by EFSA

1) On 25/03/2019 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of dried fruits of *Synsepalum dulcificum* as a novel food. Ref. Ares(2019)2068055 – 25/03/2019.
2) On 26/03/2019, a valid application on the safety of dried fruits of *Synsepalum dulcificum* as a novel food, which was submitted by Baïa Food Co. (Medicinal Gardens S.L.), was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2018/0709) and the scientific evaluation procedure was initiated.
3) On 18/10/2019, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 08/07/2020, additional information was provided by the applicant through the Commission e-submission portal.
5) On 22/07/2020 and 14/08/2020, EFSA requested the applicant to provide further clarifications to the additional information provided.
6) On 27/07/2020 and 17/03/2021, additional clarifications were provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) During its meeting on 27/04/2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of dried fruits of *Synsepalum dulcificum* as a NF pursuant to Regulation (EU) 2015/2283.

References

Aguiló Aguayo I and Echeverria Cortada G, 2018. Evaluation of the thermal treatment on the puree obtained from the fruit Synsepalum dulcificum and summary of activities on trained sensorial panel. Unpublished report, IRTA.

Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ, 1990. Basic local alignment search tool. Journal of Molecular Biology, 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Andrade AC, Martins MB, Rodrigues JF, Coelho SB, Pinheiro ACM and Bastos SC, 2019. Effect of different quantities of miracle fruit on sour and bitter beverages. LWT, 99, 89–97. https://doi.org/10.1016/j.lwt.2018.09.054
Beidler LM and Smallman RL, 1965. Renewal of cells within taste buds. J. Cell Bio., 27, 263–272.
Brehler R, Theissen U, Mohr C and Luger T, 1997. "Latex-fruit syndrome": frequency of cross-reacting IgE antibodies. Allergy, 52, 404–410.
Capitanio A, Lucci G and Tommasi L, 2011. Mixing taste illusions: the effect of miraculin on binary and trinary mixtures. Journal of Sensory Studies, 26, 54–61. https://doi.org/10.1111/j.1745-459X.2010.00321.x
CERETOX (Centre de Recerca en Toxicologia), 2018a. Study IF-74616: Genotoxicity assay of a lyophilized food on bacterial by Bacterial Reverse Mutation Test. Unpublished report.
CERETOX (Centre de Recerca en Toxicologia), 2018b. Study No. IF-74516: genotoxicity study of a novel food: micronucleus test in rats. Unpublished report.
CERETOX (Centre de Recerca en Toxicologia), 2018c. Study No. IF-81517: Acute Oral Toxicity of a novel food by Up-and-Down-Procedure (UDP) (OECD 425) in female Wistar rats. Unpublished report.
CERETOX (Centre de Recerca en Toxicologia), 2018d. Study No. 73416: 90-Day Oral Toxicity Study of a Lyophilized Novel Food in Wistar Rats with a 14-day Recovery Period. Unpublished report.
Charles River, 2020a. Study No. 20229053: Evaluation of the Mutagenic Activity of Dried fruits of *Synsepalum dulcificum* (Miracle Berry Freeze-Dried Powder) in the Bacterial Reverse Mutation Test (Plate Incorporation and Pre-Incubation Methods). Unpublished report.
Charles River, 2020b. Study code: 20/020-013C: Dried fruits of *Synsepalum dulcificum* (Miracle Berry Freeze-Dried Powder): A GLP *In Vitro* Mammalian Cell Micronucleus Test. Unpublished report.
Cózar Fernández B, 2018. No title. Department of Biotechnology and Plant Biology, Higher Tehcnical School of Agronomic Engineering, Food Science and Biosystems. Unpublished report.
Dalzel A, 1967. The History of Dahomy; an Inland Kingdom of Africa. T. Spilsbury and son, London, 230 pp.
De Faria AD, Pirani JR, Ribeiro JELD, Nylinder S, Terra-Araujo MH, Vieira PP and Swenson U, 2017. Towards a natural classification of Sapotaceae subfamily Chrysophylloideae in the Neotropics. Botanical Journal of the Linnean Society, 185, 27–55.
De Souza APA, Nascimento LMAM, De Lima VCO, De Carvalho FMC, Dos Santos EA and De Araujo Morais AH, 2017. Anti-tryptic activity in seed and food product of chia (*Salvia hispanica* L.). Demetra, 12, 319–331. https://doi.org/10.12957/demetra.2017.25636
EFSA (European Food Safety Authority), 2018. Technical Report on the notification of decorticated grains of *Digitaria exilis* (Kippist) Stapf as a traditional food from a third country pursuant to Article 14 of Regulation (EU) 2015/2283. EFSA supporting publication 2018;EN-1443, 9 pp. https://doi.org/10.2903/sp.efsa.2018.EN-1443
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
EFSA Scientific Committee, 2011. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. https://doi.org/10.2903/j.efsa.2011.2379
Guillamon E, Pedrosa MM, Burbano C, Cuadrado C, de Cortes Sanchez M and Muzquiz M, 2008. The trypsin inhibitors present in seed of different grain legume species and cultivar. Food Chemistry, 107, 68–74. https://doi.org/10.1016/j.foodchem.2007.07.029
Hudson SD, Sims CA, Odabasi AZ, Colquhoun TA, Snyder DJ, Stamps JJ, Dotson SC, Puentes L and Bartoshuk LM, 2018. Flavor alterations associated with miracle fruit and gymnema sylvestre. Chemical Senses, 43, 481–488. https://doi.org/10.1093/chemse/bjy032
Igarashi G, Higuchi R, Yamazaki T, Ito N, Ashida I and Miyaoka Y, 2013. Differential sweetness of commercial sour liquids elicited by miracle fruit in healthy young adults. Food Science and Technology International, 19, 243–249. https://doi.org/10.1177/1082013212443060
Juhé-Beaulaton D, 2014. Le fruit miracle (Synsepalum dulcificum) : des voyageurs sur la côte ouest africaine aux laboratoires pharmaceutiques. Proceedings of the Journées d'études "Les collections de Muséums comme sources historiques: Savoirs naturalistes, dynamiques environnementales", Paris, France, 2014-11-13.
Kurihara K and Beidler LM, 1968. Taste-modifying protein from miracle fruit. Science, 161, 1241–1243. https://doi.org/10.1126/science.161.3847.1241
Labat J-B, 1730. Voyage du Chevalier des Marchais en Guinée, Isles Voisines, et à Cayenne, Fait en 1725, 1726 & 1727. Par le R. Père Labat, de l'Ordre des Frères Prêcheurs, Aux dépens de la Compagnie, Amsterdam, 1480 pp.
Lei DK and Grammer LC, 2019. An overview of allergens. Allergy Asthma Proc., 40, 362–365. https://doi.org/10.2500/aap.2019.40.4247
Masuda Y, Nirasawa S, Nakaya K and Kurihara Y, 1995. Cloning and sequencing of a cDNA encoding a taste-modifying protein miraculin. Gene, 161, 175–177. https://doi.org/10.1016/0378-1119(95)00198-f
Menéndez-Rey A, 2018. No title. Department of Biotechnology and Plant Biology, Higher Tehcnical School of Agronomic Engineering. Food Science and Biosystems. Unpublished report.
Menéndez-Rey A, González-Martos R, Peng Y, Quiroz-Troncoso J, Alegría-Aravena N, Sánchez-Díez M, Maestu-Unturbe C, Bensadon-Naeder L and Ramírez-Castillejo C, 2021. Quantification of lectins in *Synsepalum dulcificum* and comparison with reference foods. Food Chemistry, 352, 129341. https://doi.org/10.1016/j.foodchem.2021.129341

Njoku NE, Ubbaonu CN, Alagbaoso SO, Agunwah IM and Eluchie CN, 2016. Proximate, Anti-Nutritional and Phytochemical Composition of the Yellow Variety of the Synsepalum Dulcificum (Miracle Fruit) Berry. https://doi.org/10.12691/ajfst-4-4-3.

OECD (Organisation for Economic Co-operation and Development), 1997. OECD Guideline for Testing of Chemicals: Bacterial Reverse Mutation Test 471. Available at: https://www.oecd.org/chemicalsafety/risk-assessment/1948418.pdf

OECD (Organisation for Economic Co-operation and Development), 1998. OECD Guideline for Testing of Chemicals: Repeated Dose 90-day Oral Toxicity Study in Rodents, Test 480. Available online: https://www.oecd.org/env/ehs/testing/E408_1998.PDF

OECD (Organisation for Economic Co-operation and Development), 2008. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264071049-en

OECD (Organisation for Economic Co-operation and Development), 2016a. Test No. 476: *In Vitro* Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264809-en

OECD (Organisation for Economic Co-operation and Development), 2016b. Test No. 474: Mammalian Erythrocyte Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. https://doi.org/10.1787/9789264264762-en

OECD (Organisation for Economic Co-operation and Development), 2016c. 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

Perea-Martinez I, Nagai T and Chaudhari N, 2013. Functional cell types in taste buds have distinct longevities. PLoS ONE, 8. https://doi.org/10.1371/journal.pone.0053399

Rodrigues JF, da Silva Andrade R, Carvalho Bastos S, Bragança Coelho S and Marques Pinheiro AC, 2016. Miracle fruit: an alternative sugar substitute in sour beverages. Appetite, 107, 645–653. https://doi.org/10.1016/j.appet.2016.09.014

Sanematsu K, Kitagawa M, Yoshida R, Mirasawa S, Shigemura N and Ninomiya Y, 2016. Intracellular acidification is required for full activation of the sweet taste receptor by miraculin. Scientific Reports, 6, 22807. https://doi.org/10.1038/srep22807

Tafazoli S, Vo TD, Roberts A, Rodriguez C, Viñas R, Madonna ME, Chiang YH, Noronha JW, Holguin JC, Ryder JA and Perlstein A, 2019. Safety assessment of miraculin using *in silico* and *in vitro* digestibility analyses. Food and Chemical Toxicology, 133, 110762. https://doi.org/10.1016/j.fct.2019.110762. Epub 2019 Aug 14. Erratum in: Food Chem Toxicol. 2020 Mar;137:111136. PMID: 31421212.

Tafazoli S, Vo TD, Roberts A, Rodriguez C, Viñas R, Madonna ME, Chiang YH, Noronha JW, Holguin JC, Ryder JA and Perlstein A, 2020. Corrigendum to "Safety assessment of miraculin using *in silico* and *in vitro* digestibility analyses". [Food Chem. Toxicol. 133 (2019 Nov) 110762]. Food Chem Toxicol. 2020 Mar;137:111136. https://doi.org/10.1016/j.fct.2020.111136. Epub 2020 Jan 28. Erratum for: Food Chem Toxicol. 2019 Nov;133:110762.

Takai A, Satoh M, Matsuyama T, Ito A, Nakata R, Aoyama T and Inoue H, 2013. Secretion of miraculin through the function of a signal peptide conserved in the Kunitz-type soybean trypsin inhibitor family. FEBS Letters, 587, 1767–1772.

Theerasilp S, Hitotsuya H, Nakajo S, Nakaya K, Nakamura Y and Kurihara Y, 1989. Complete amino acid sequence and structure characterization of the taste-modifying protein, miraculin. Journal of Biological Chemistry, 264, 6655–6659.

Wilken MK and Satiroff BA, 2012. Pilot study of "miracle fruit" to improve food palatability for patients receiving chemotherapy. Clin J Oncol Nurs, 16, E173–E177. https://doi.org/10.1188/12.CJON.E173-E177

Wong JM and Kern M, 2011. Miracle fruit improves sweetness of a low-calorie dessert without promoting subsequent energy compensation. Appetite, 56, 163–166. https://doi.org/10.1016/j.appet.2010.10.005

Woodfolk JA, Commins SP, Schuyler AJ, Erwin EA and Platts-Mills TAE, 2015. Allergens, sources, particles, and molecules: why do we make IgE responses? Allergology International, 64, 295–303. https://doi.org/10.1016/j.alit.2015.06.001

Abbreviations

AA	aminoanthracene
ADME	absorption, distribution, metabolism and excretion
BLAST	Basic Local Alignment Search Tool
bw	body weight
CERETOX	Centre de Recerca en Toxicologia
CFU	colony forming units
CMC	carboxymethylcellulose
DMB	dried miracle berries
DNAse	deoxyribonuclease

dw	dry weight
ELISA	enzyme-linked immunosorbent assay
FAERS	US FDA adverse event reporting system
FDA	Food and Drug Administration
FID	flame ionisation detector
GAE	Gallic acid equivalents
GC	gas chromatography
GI	gastrointestinal
GLP	Good Laboratory Practice
GRAS	generally recognized as safe
HACCP	Hazard Analysis Critical Control Points
HPLC	high-performance liquid chromatography
ICP	inductively Coupled Plasma
KTi	Kunitz trypsin inhibitor
LB	lower bound
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LOQ	limit of quantification
MAS	metabolic activation system
MN	micronuclei
MOE	Margin of Exposure
MS	mass spectrometry
NC	negative control
NCBI	National Center for Biotechnology Information
NDA Panel	Panel on Nutrition, Novel Foods and Food Allergens
NF	Novel Food
OECD	Organisation for Economic Co-operation and Development
OES	optical emission spectrometry
PAHs	polycyclic aromatic hydrocarbons
PC	positive control
PCE	polychromatic erythrocytes
PCR	polymerase chain reaction
qH-NMR	quantitative ^{1}H-nuclear magnetic resonance
RH	relative humidity
RNAse	ribonuclease
STI	soybean trypsin inhibitor
TIU	trypsin inhibitor unit
UFLC	ultra-fast liquid chromatography
UPD	up-and-down
USDA	United States Department of Agriculture
UV–VIS	ultraviolet–visible
w/w	weight per weight

Annex A – Summary of the 90-day oral toxicity study (OECD TG 408, limit test)

Study title	**90-day oral toxicity study of a lyophilized novel food in Wistar rats with a 14-day recovery period (CERETOX, 2018d)**
Tested system	Wistar rats (RjHan:Wistar)
Test material	The novel food, batch #1.
Dose/concentration (route of administration)	Control group: 0 (vehicle: phosphate buffer solution of carboxymethylcellulose (CMC) sodium salt) 10 animals/sex Recovery control group: 5 animals/sex Dose group: 2,000 mg/kg bw/day (lyophilized powder of miracle berries (MBP) suspended in the vehicle) 10 animals/sex Recovery dose group: 5 animals/sex
Method	OECD TG 408 (limit test)

Key results

		Dose groups (mean ± SD)					
Parameter (unit)	**Sex**	**0 (control); *0 (recovery)***	**2,000 (main); *2,000 (recovery)***	**0 (control); *0 (recovery)***	**2,000 (main); *2,000 (recovery)***	***0 (recovery)***	***2,000 (recovery)***
		At day 45		**At day 90**		***At day 105***	
Haematology							
RBC (x10^{12}/L)	M	7.53 (0.47)	7.70 (0.69)	7.97 (0.61)	8.09 (0.22)	*7.42 (0.43)*	*7.26 (1.38)*
		7.93 (0.20)	*7.75 (0.32)*	*8.54 (0.66)*	*9.40 (0.31)**		
	F	7.45 (0.25)	7.85 (0.49)*	7.52 (0.24)	7.77 (0.74)	*7.65 (0.76)*	*7.84 (0.73)*
		8.02 (0.71)	*8.41 (0.86)*	*8.24 (0.42)*	*7.80 (1.99)*		
MCV (fL)	M	61.17 (2.02)	58.97 (1.52)*	56.83 (4.38)	57.33 (2.89)	*55.34 (2.31)*	*56.12 (2.08)*
		58.85 (0.46)	*60.49 (0.87)***	*58.94 (1.13)*	*58.93 (0.39)*		
	F	62.95 (1.82)	59.43 (4.91)*	60.98 (3.15)	57.92 (5.61)	*56.31 (8.56)*	*50.79 (7.37)*
		55.61 (7.96)	*53.52 (9.07)*	*57.86 (9.39)*	*56.13 (9.08)*		
MCH (g/L)	M	19.35 (0.63)	17.72 (2.83)	18.38 (0.98)	18.39 (0.63)	*18.08 (0.57)*	*18.01 (1.29)*
		18.76 (0.54)	*19.03 (0.25)*	*18.37 (0.55)*	*18.60 (0.56)*		
	F	19.50 (0.59)	18.69 (1.47)*	19.06 (0.57)	18.73 (1.63)	*18.06 (2.59)*	*17.48 (2.34)*
		17.85 (2.37)	*17.49 (2.68)*	*17.79 (3.19)*	*17.17 (2.40)*		
BNE (%)	M	2.6 (2.3)	0.3 (0.7)**	1.5 (1.1)	0.2 (0.4)**	*0.2 (0.4)*	*0.2 (0.4)*
		1.0 (1.4)	*0.6 (0.9)*	*0.0 (0.0)*	*0.0 (0.0)*		
	F	0.5 (0.5)	0.7 (1.1)	0.7 (1.1)	0.5 (0.7)	*0.2 (0.4)*	*0.2 (0.4)*
		0.6 (0.9)	*0.0 (0.0)*	*0.4 (0.5)*	*0.8 (0.8)*		

Parameter (unit)	Sex	Dose groups (mean ± SD)					
		0 (control); *0 (recovery)*	**2,000 (main); *2,000 (recovery)***	**0 (control); *0 (recovery)***	**2,000 (main); *2,000 (recovery)***	***0 (recovery)***	***2,000 (recovery)***
		At day 45		**At day 90**		***At day 105***	
MCHC (g/L)	M	316.44 (4.80)	299.76 (43.46)	324.30 (14.73)	321.36 (16.86)	*327.38 (21.03)*	*320.81 (16.70)*
		318.70 (6.92)	*314.65 (4.08)*	*311.73 (6.91)*	*315.54 (7.68)*		
	F	309.79 (7.94)	314.66 (5.80)	312.96 (8.40)	323.79 (11.17)*	*321.69 (17.80)*	*344.69 (13.25)**
		321.61 (10.15)	*327.72 (15.31)*	*306.69 (6.32)*	*306.98 (9.76)*		
MON (%)	M	7.7 (2.9)	9.6 (4.1)	0.8 (0.9)	0.1 (0.3)*	*4.2 (2.4)*	*5.0 (3.0)*
		7.4 (4.0)	*7.8 (2.8)*	*2.0 (0.7)*	*2.6 (1.7)*		
	F	5.7 (2.1)	5.7 (3.1)	0.3 (0.9)	0.0 (0.0)	*6.2 (3.0)*	*5.0 (1.6)*
		5.6 (1.1)	*5.6 (2.1)*	*2.0 (1.6)*	*3.0 (1.0)*		
EOS (%)	M	1.3 (1.3)	0.8 (1.3)	1.1 (0.9)	3.2 (1.6)**	*0.8 (1.3)*	*0.8 (1.1)*
		2.2 (2.5)	*1.6 (2.6)*	*2.0 (2.0)*	*0.4 (0.5)*		
	F	0.6 (0.7)	1.1 (2.5)	1.7 (1.4)	2.2 (2.0)	*0.4 (0.5)*	*1.8 (1.9)*
		0.8 (0.8)	*1.4 (0.5)*	*0.6 (0.9)*	*1.2 (0.8)*		
Coagulation parameters							
PT (sec)	M	14.4 (0.8)	15.7 (1.5)*	14.8 (0.9)	15.7 (0.9)*	*17.5 (5.1)*	*13.4 (0.9)**
		15.0 (0.6)	*16.1 (1.5)*	*26.5 (7.7)*	*37.9 (12.4)*		
	F	15.5 (1.3)	16.0 (1.1)	15.4 (1.3)	15.9 (2.9)	*11.7 (0.3)*	*12.5 (0.9)*
		–	*16.4 (1.4)*	*36.6 (17.4)*	*30.3 (9.0)*		
APTT (sec)	M	47.0 (3.6)	49.9 (4.0)*	34.8 (9.1)	41.9 (13.4)	*47.5 (25.6)*	*34.1 (12.9)*
		46.3 (13.1)	*48.5 (2.8)*	*87.9 (–)*	*51.8 (29.4)*		
	F	62.2 (6.6)	49.2 (4.5)*	30.8 (6.1)	45.5 (27.8)	*32.4 (6.6)*	*33.2 (9.2)*
		43.4 (2.9)	*47.8 (4.8)*	*48.8 (10.5)*	*82.6 (26.5)**		
Clinical blood chemistry							
TRIGL (mg/dL)	M	289 (67)	292 (72)	239 (101)	183 (46)	*192 (83)*	*231 (78)*
		341 (66)	*266 (32)*	*279 (93)*	*287 (39)*		
	F	168 (21)	133 (43)*	181 (60)	190 (67)	*202 (76)*	*231 (97)*
		186 (89)	*156 (33)*	*229 (56)*	*234 (60)*		

Parameter (unit)	Sex	Dose groups (mean ± SD)					
		0 (control); *0 (recovery)*	**2,000 (main); *2,000 (recovery)***	**0 (control); *0 (recovery)***	**2,000 (main); *2,000 (recovery)***	***0 (recovery)***	***2,000 (recovery)***
		At day 45		**At day 90**		***At day 105***	
AST/GOT (U/L)	M	136 (31)	184 (61)*	188 (92)	121 (34)	*155 (55)*	*140 (28)*
		132 (37)	*138 (14)*	*180 (51)*	*180 (23)*		
	F	121 (25)	105 (28)	142 (51)	104 (53)	*174 (73)*	*125 (35)*
		99 (17)	*111 (30)*	*150 (17)*	*126 (14)*		
CK (U/L)	M	315 (106)	705 (412)*	1,113 (434)	722 (322)*	*954 (234)*	*999 (506)*
		323 (146)	*482 (186)*	*1,293 (352)*	*1,654 (851)*		
	F	379 (246)	298 (112)	930 (256)	461 (183)**	*908 (599)*	*599 (240)*
		291 (124)	*266 (106)*	*1,211 (142)*	*832 (180)**		
T-CHOL (mg/dL)	M	173 (37)	152 (37)	147 (44)	102 (20)*	*113 (27)*	*97 (10)*
		151 (29)	*124 (18)*	*148 (37)*	*109 (5)*		
	F	122 (13)	112 (13)	107 (21)	101 (25)	*100 (16)*	*97 (16)*
		107 (26)	*106 (20)*	*127 (21)*	*111 (17)*		
ALT/GPT (U/L)	M	93 (31)	110 (26)	126 (70)	75 (33)	*136 (40)*	*134 (26)*
		123 (45)	*71 (9)**	*89 (21)*	*150 (28)***		
	F	61 (10)	92 (38)	109 (36)	77 (28)*	*121 (36)*	*122 (32)*
		44 (14)	*99 (26)***	*134 (16)*	*142 (12)*		
T-BIL (mg/dL)	M	0.72 (14)	0.67 (0.12)	0.38 (0.15)	0.24 (0.12)*	*0.44 (0.09)*	*0.33 (0.10)*
		0.64 (0.17)	*0.69 (0.08)*	*0.81 (0.33)*	*0.85 (0.18)*		
	F	0.40 (0.13)	0.41 (0.14)	0.34 (0.12)	0.18 (0.10)**	*0.52 (0.16)*	*0.44 (0.16)*
		0.43 (0.16)	*0.34 (0.19)*	*0.66 (0.16)*	*0.55 (0.23)*		
ALKP (U/L)	M	419 (105)	421 (116)	249 (120)	190 (45)	*206 (18)*	*132 (18)*
		454 (83)	*285 (38)***	*263 (18)*	*215 (38)**		
	F	255 (38)	216 (39)*	139 (29)	126 (27)	*169 (39)*	*130 (18)*
		198 (52)	*212 (47)*	*177 (28)*	*185 (45)*		
UREA (mg/dL)	M	67 (20)	67 (15)	50 (12)	40 (3)*	*37 (2)*	*41 (2)**
		62 (13)	*49 (2)*	*42 (2)*	*43 (3)*		
	F	42 (5)	44 (4)	40 (4)	38 (5)	*44 (8)*	*43 (3)*
		48 (7)	*44 (2)*	*41 (11)*	*38 (3)*		

Parameter (unit)	Sex	Dose groups (mean ± SD)					
		0 (control); *0 (recovery)*	2,000 (main); *2,000 (recovery)*	0 (control); *0 (recovery)*	2,000 (main); *2,000 (recovery)*	*0 (recovery)*	*2,000 (recovery)*
		At day 45		At day 90		At day 105	
K (mmol/L)	M	8.20 (0.46)	8.51 (0.77)	5.72 (1.21)	4.81 (0.59)*	*5.55 (0.73)*	*5.63 (0.19)*
		7.56 (0.83)	*6.82 (0.12)*	*5.84 (0.74)*	*6.37 (0.23)*		
	F	5.51 (0.44)	5.46 (0.59)	4.88 (0.58)	4.50 (0.58)*	*5.31 (0.34)*	*5.06 (0.36)*
		5.73 (0.25)	*5.27 (0.69)*	*6.03 (0.74)*	*5.50 (0.26)*		
Ca (mmol/L)	M	3.43 (0.50)	3.46 (0.79)	3.13 (1.01)	2.51 (0.15)	*2.75 (0.11)*	*2.78 (0.20)*
		3.59 (0.20)	*2.96 (0.27)***	*2.56 (0.14)*	*2.69 (0.14)*		
	F	3.10 (0.22)	2.47 (0.22)**	2.79 (0.46)	2.77 (0.27)	*2.94 (0.18)*	*2.92 (0.19)*
		2.36 (0.82)	*2.48 (0.27)*	*2.78 (0.47)*	*3.22 (0.30)*		
P (mmol/L)	M	2.52 (0.28)	2.38 (0.26)	3.11 (0.98)	2.40 (0.32)*	*3.11 (0.15)*	*2.70 (0.30)**
		2.56 (0.28)	*2.28 (0.11)*	*2.85 (0.32)*	*2.49 (0.37)*		
	F	2.04 (0.20)	2.34 (0.23)**	2.47 (0.46)	2.29 (0.30)	*3.43 (0.08)*	*2.98 (0.28)***
		2.25 (0.18)	*2.43 (0.17)*	*2.51 (0.43)*	*2.31 (0.29)*		
Organ weight (g)							
Liver	M	–	–	19.8 (3.2)	17.4 (2.5)	*19.1 (3.0)*	*17.4 (2.0)*
	F	–	–	10.2 (0.5)	8.0 (3.0)**	*9.5 (1.3)*	*9.3 (1.1)*
Spleen	M	–	–	1.1 (0.1)	0.9 (0.1)**	*1.0 (0.1)*	*1.2 (0.1)*
	F	–	–	0.6 (0.2)	0.6 (0.2)	*0.6 (0.1)*	*0.7 (0.0)*
Organ weight/bw ratio (%)							
Brain	M	–	–	0.36 (0.03)	0.40 (0.03)*	*0.34 (0.02)*	*0.37 (0.02)**
	F	–	–	0.62 (0.06)	0.63 (0.03)	*0.64 (0.08)*	*0.60 (0.05)*
Liver	M	–	–	3.72 (0.47)	3.39 (0.37)	*3.44 (0.37)*	*3.11 (0.29)*
	F	–	–	3.38 (0.21)	2.99 (0.27)**	*3.06 (0.16)*	*2.95 (0.09)*

RBC: erythrocyte count; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; BNE: band neutrophils; MCHC: mean corpuscular haemoglobin concentration; MON: monocytes; EOS: eosinophils; PT: prothrombin time; APTT: activated partial thromboplastin time; TRIGL: triglycerides; AST/GOT: aspartate aminotransferase; CK: creatine kinase; ALKP: alkaline phosphatase; T-CHOL: cholesterol, total; ALT/GPT: alanine aminotransferase; T-BIL: bilirubin, total.
*: Significantly different from control ($p < 0.05$).
**: Significantly different from control ($p < 0.01$).