REGULATED PRODUCTS SAFETY ASSESSMENT

Safety Assessment of Dried Miracle Berry (*Synsepalum Dulcificum*) as a Novel Food for Use in Food and Food Supplements (RP1351)

Food Standards Agency[1], Food Standards Scotland[2]

[1] Regulated Products Risk Assessment, Food Standards Agency, UK, [2] Risk Assessment, Food Standards Scotland, UK

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FSA Research and Evidence

The Food Standards Agency (FSA) and Food Standards Scotland (FSS) received an application from Baïa Food Co. ("the applicant") for the authorisation of Dried Miracle Berry (*Synsepalum dulcificum*) also referred to as DMB, as a novel food in November 2021.

The novel food is produced from drying the pitted fruits (pulp and skin) of *Synsepalum dulcificum*. The dried mass is then milled into a powder.

This new application is seeking to use the novel food as a functional food supplement to modify the taste of sour foods due to the presence of miraculin. The target population is the general population, excluding pregnant and lactating women and children.

To support the FSA and FSS in their evaluation of the application, the Advisory Committee on Novel Foods and Processes (ACNFP) were asked to review the safety dossier and supplementary information provided by the applicant. The views of the Committee were taken into account by the FSA and FSS, who concluded that the applicant had provided sufficient information to assure the novel food, Dried Miracle Berry, was safe under the proposed conditions of use. The anticipated intake levels and the intended use in food and food supplements was not considered to be nutritionally disadvantageous.

This safety assessment represents the opinion of the FSA and FSS.

This is a joint FSA and FSS publication.

1. Introduction

In November 2021, Baïa Food Co ("the applicant") submitted a full novel food application for the authorisation of Dried Miracle Berry. The novel food produced by drying the pitted fruit of the *Synsepalum dulcificum* species is intended to be used as a food supplement. The target population is the general population, excluding pregnant and lactating women and children.

The FSA and FSS have undertaken a safety assessment for Dried Miracle Berry under the novel foods legislation, assimilated Regulation (EU) 2015/2283. To support the safety assessment, the ACNFP provided the advice outlined in this opinion to the FSA and FSS.

The evaluation by the ACNFP assessed the food safety risks of the novel food and its production, in line with Article 7 of assimilated Commission Implementing Regulation (EU) 2017/2469. The regulatory framework and the technical guidance put in place by the European Food Safety Agency (EFSA) for full novel food applications is retained as the basis and structure for the assessment (EFSA NDA Panel, 2016).

Following the review by the ACNFP in April and November 2023 as well as in September 2024, further information was sought from the applicant on production process, composition and specifications, ADME (Absorption, Distribution, Metabolism & Excretion), nutrition and allergenicity. The final advice from the Committee was agreed in February 2025, allowing the FSA and FSS to complete the risk assessment.

The document outlines the conclusions of the FSA and FSS on the safety of Dried Miracle Berry as a novel food. This represents the opinions of the FSA and FSS.

2. Assessment

2.1. Identity of the novel food

The novel food is fruit from the miracle fruit shrub *Synsepalum dulcificum* and is a member of the *Sapotaceae* family. The applicant refers to this novel food as Dried Miracle Berry, hereafter referred to as DMB. *Synsepalum dulcificum* is native to the tropical forest regions of West Africa. The novel food under this application is farmed in Ghana and consists of fruits from *S. dulcificum* that are pitted and then dried whole (pulp with skin).

The main constituent that acts as a taste modifier is miraculin, a protein consisting of amino acids belonging to the Kunitz-type soybean trypsin inhibitor family. It is noted that this trypsin inhibitor has lost its activity in the novel food (Takai et al., 2013). It has a molecular weight of 24.6 kDa of which up to 13.9% is represented by sugar moieties, including glucosamine (31%), mannose (30%), fucose (22%), xylose (10%) and galactose (7%) (Theerasilp et al., 1989).

Presence of miraculin was established from in-house analysis. The information supplied indicated that the Nuclear Magnetic Resonance based method could quantify the compound with levels ranging from 1.7% - 2.1% of the total weight of the novel food. The conclusion reached was that while the method was qualitative, its ability to quantify miraculin had not been demonstrated. Sensory studies were also undertaken to investigate the effectiveness of miraculin's taste modifying capacity on unsweetened sour foods.

The identity of the novel food was considered to be appropriately characterised.

2.2. Production Process

The pitted fruit of *S. dulcificum* is the main raw ingredient in DMB's production. The processing is carried out under controlled environmental conditions and within the principles of HACCP (Hazard Analysis and Critical Control Points).

The fruits are manually harvested in Ghana from mature cultivated plants, sorted, cooled and sent for processing. They are then sorted again and rinsed in tap water. The free chlorine level of the water is tested to ensure that chlorate residues are kept as low as practically possible. These were analysed in the novel food with amounts up to 0.046 mg/kg. This is not above the maximum residue level for berries and small fruits of 0.05 mg/kg (EC, 2020). The fruits then undergo mechanical pitting (removing the stone).

The pitted fruits are blended into a puree whole, with skin on, together with anti-caking agents. This puree is then dried by lyophilisation, a method aimed at reducing the moisture to levels of around 1%-2% without significantly changing heat sensitive constituents, namely miraculin. The resulting dry 'cake' is milled to a powder, packaged then stored.

Controls in the production process and how this impacted on variability of the final product, especially on mycotoxin levels, was queried. The main source of variability was identified as the impact of different growing seasons and climate conditions. Evidence was presented showing that mycotoxins were not detected, and microbiological parameters were below maximum permitted levels, providing assurance that factors impacting hazards were identified and managed in the food safety management system.

The production process has characterised the potential hazards and the corresponding control measures are appropriate.

2.3. Compositional information

Results from five independently manufactured batches of DMB were provided. Table 1 summarises proximate analysis for the novel food. Analysis was performed by accredited laboratories with certifications provided. Where in-house analysis was utilised, full methodology and supporting validation documentation were provided. These demonstrate the characteristics of the novel food, and the effective management of any hazards identified.

Three batches of the novel food were analysed to determine the total polyphenolic content using spectrophotometry and data provided in the original application. Following a request for the classes of the polyphenols present, further information on the concentration of the phenolic compounds according to the extraction method, in comparison with those from reference foods were provided (Table 2).

The main phenolic compounds that were present in the novel food were identified by HPLC (high-performance liquid chromatography). The concentrations were found to be consistent with similar foods. It was also argued that the proposed use for the novel food is small at 0.9 g/day, hence the ingested amount of these compounds is lower in comparison to other consumed foods rich in phenolic compounds.

Table 1. Proximate analysis of the novel food

Parameter	Method	Specification	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Mean
Moisture (g/100 g)	Gravimetric	< 6 g/100 g	2.57	5.27	3.73	5.2	5.2	4.39
Ash (g/100 g)	Gravimetric	3.5 – 8.5 g/100 g	3.7	3.53	4.23	5.00	5.4	4.37
Total carbohydrate (g/100 g)	Calculation	70 – 87 g/100 g	86.3	81.0	77.7	79.4	80.6	81.0
Sugars (g/100 g)	Ion chromatography/ Pulsed amperometry	50 – 75 g/100 g	72.5	66	64.9	56.5	67.2	65.4
Total protein (g/100 g)	Kjeldahl	3.5 – 6.0 g/100 g	5.03	5.1	6.0	5.3	4.2	5.13
Total Fat (g/100 g)	Gravimetric	0.5 – 3.5	2.47	3.2	2.73	<0.5	1.8	<0.5-3.2
Saturated fatty acids (g/100 g)	GC-FID	-	1.1	1.33	0.7	<0.1	0.8	<0.1-1.33
Sodium Chloride (g/100 g)	ICP-MS	0.01-0.06 g/110 g	0.029	0.021	0.036	0.043	0.037	0.033
Fibre (g/100 g)	Gravimetric	1 – 6.5 g/100 g	-	1.9	5.6	5.1	2.8	3.85
Energy (kJ/100 g)	Calculation	1500-1600 kJ/100 g	1619	1597	1569	1481	1531	1559
Energy (kcal/100 g)	Calculation	350-390 kcal/100 g	387	377	371	349	361	369

GC-FID = gas chromatography – flame ionisation detection, ICP-MS = inductively-coupled plasma mass spectrometry

Table 2. Phenolic concentrations in the novel food in comparison to reference foods

Compound	Concentration (µg/g DW) in Novel Food				Reference foods	
	Aqueous extraction	Low ethanol	Low aqueous	Ethanol	Concentration (µg/g FW)	Examples
Gallic acid	174	54.5	42.8	37.7	1.6 - 46.7	Date, blackberry, cloudberry, grapefruit, banana
Protocatechuic acid	771	570.7	386.6	119.5	0.1 - 217.9	Chicory, eggplant, onion, almond, grapefruit, date, apple, kiwi, pomegranate
Catechin	145	70.8	70	25.4	3 – 1,078	Cocoa powder, blackberry, raspberry, strawberry, apricot, peach, apple, pear, banana, pomegranate
Caffeic acid	0	3.75	2.5	0	0.02 – 1,411	Plum, date, cranberry, grapefruit, peach, apple, pear
Syringic acid	2.17	40.1	22.2	12.1	9 - 60.6	Date, apple, grape, cauliflower, walnut
Rutin (Quercetin 3-O-rutinoside)	0	0	0	0.5	0.04 - 190	Plum, blackberry, raspberry, apricot, apple, pear, tomato, lettuce
Vanillin	0.36	6.9	2.16	3.2	0.2 - 1	Cocoa powder, olive
Coumaric acid	9.49	12.7	9.6	5.58	0.04 - 57.7	Plum, date, cranberry, strawberry, grapefruit, apple, pear
Ferulic acid	4.3	7.49	5.73	2.42	0.1 - 118.3	Date, cranberry, grapefruit, apple, eggplant, olive
Salicylic acid (4-Hydroxybenzoic acid)	2.7	5.26	3.4	3.8	0.05 - 46.6	Cranberry, grapefruit, date, loquat
Quercetin	2.92	8.17	8.36	6.7	0.2 - 420	Bilberry, elderberry, cranberry, raspberry, apple, onion
Cinnamic acid	0.32	0.55	0.38	0	0.2 - 41.2	Cranberry, strawberry, orange, olive

FW = fresh weight.

An in-house method for quantification of miraculin based on proton nuclear magnetic resonance (1H-NMR) analysis was presented in the dossier. Further information was sought on the choice of method selected. The justification provided indicated that this method was demonstrated to be reproducible for the quantification of miraculin, as there was no other known method for miraculin quantification previously standardised for food analysis. The further information provided evidenced specificity of the method for miraculin. However, there was a lack of information following several requests, on how it was assured that miraculin could be accurately quantified to give confidence in the quality of the analysis. It was noted that the glycosylation pattern of miraculin would impact the accuracy of the information. From the data supplied the quantification of miraculin could not be confirmed but would be expected to be low given the proposed use level is 0.9 g/day.

Heavy metal levels were analysed and compared to established EU limits (Table 3). The method of analysis was inductively coupled plasma mass spectrometry (ICP-MS). All analyses were below EU limits.

Table 3. Heavy metals analysis for the novel food

Parameter	Lot 1	Lot 2	Lot. 3	Lot 4	Lot 5	Mean
Arsenic (mg/kg)	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Cadmium (mg/kg)	0.069	0.063	0.14	0.088	0.070	0.086
Mercury (mg/kg)	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Lead (mg/kg)	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04

A Certificate of Analysis provided for a wide range of pesticides as well as for dioxins, furans, and PCBs showed that no residues were detected in the novel food. A summary of the levels of polycyclic aromatic hydrocarbons (PAH) analysed using gas chromatography–mass spectrometry (GC-MS) method and or ultra-fast liquid chromatography (UFLC) was provided; none were found above 0.71 µg/kg.

The applicant originally provided results for microbial and mycotoxins analysis in line with the guidance on preparation of applications. However, they later provided revised data to reflect the improvements in the effectiveness of their production process controls. Results of seven batches provided (Table 4) showed that microbial contamination was appropriately managed and that the controls were working effectively.

The presence of mycotoxins was measured in three batches of the novel food (Table 5) and were all under the limit of quantification of the method used (Ultra-Fast Liquid Chromatography) and below EC safety standards.

The data presented indicate the novel food and any hazards present were appropriately characterised.

Table 4. Microbial analysis of the novel food

Species	Specification in cfu/g	Lot EB 042822	Lot EB 060722	Lot EB 172522	Lot EB 191322	Lot EB 490922	Lot EB 501222	Lot EB 040323
Bacillus cereus	< 100	<10	<10	<10*	<40	<10.0	<10	<40
Clostridium Perfringens	≤ 50	<10	<10	<10	<10	<10	<10	<10
Total *Enterobacteriaceae*	< 100	<10	<10	<10	<10	<10	<10	<10
Yeasts and moulds	< 500	<100	<400	<55	<10	<10	<100	<100
Staphylococcus Coagulase +	<100	NA	NA	<10	<10	NA	NA	NA
E. coli s glucuronidase +	< 10	NA	NA	<10	<10	NA	NA	NA
Faecal Coliforms	<100	NA	NA	<10	<10	NA	NA	NA
Mycotoxins	Absence	ND	ND	NA	NA	NA	NA	ND

*Result of the counter analysis present in the COA included in Annex 2.
ND = Not detected; NA = Not analysed

Table 5. Mycotoxin analysis for of the novel food

Mycotoxins (µg/kg)	LoQ	Lot EB 042822	Lot EB 060722	Lot EB 040323
Aflatoxin B1	1	ND	ND	ND
Aflatoxin B2	1	ND	ND	ND
Aflatoxin G1	1	ND	ND	ND
Aflatoxin G2	1	ND	ND	ND
Aflatoxin B1+B2+G1+G2	1	ND	ND	ND
Fumonisin B1	1	ND	ND	ND
Fumonisin B2	1	ND	ND	ND
Fumonisins B1+ B2	25	ND	ND	ND
Ochratoxin A	1	ND	ND	ND
Zearalenone	10	ND	ND	ND
Deoxynivalenol	50	ND	ND	ND
Diacetoxyscirpenol	50	ND	ND	ND
T2 Toxins (Σ T2, HT2)	50	ND	ND	ND
Toxin HT2	50	ND	ND	ND
Toxin T2	50	ND	ND	ND
Monoacetoxyscirpenol	50	ND	ND	ND
Neosolaniol	50	ND	ND	ND
T2-triol	50	ND	ND	ND
15-Acetyl Deoxynivalenol	50	ND	ND	ND
3-Acetyl Deoxynivalenol	50	ND	ND	ND
Deoxynivalenol 3-glucoside	100	ND	ND	ND
Fusarenon X	50	ND	ND	ND
Nivalenol	50	ND	ND	ND

2.4. Stability

Stability tests on micro-organisms, sensory, moisture and pH were carried out on one batch of the novel food under representative storage conditions (20°C, relative humidity 50%) for a period of two years. Overall, the novel food remained within the specified range and supported the proposed 24-month shelf life. To ensure reproducibility in the stability of the novel food, further tests on four batches were carried out, with results demonstrating consistency with the first batch and in agreement with the 24-month shelf life proposed.

Stability tests on the novel food to temperature (–80°C, –20°C, 4°C, 20°C, 37°C and 60°C) and pH (2-20) were also conducted to determine the conditions under which miraculin (the taste modifying molecule) was denatured and inactivated. The test was undertaken using volunteers who tasted the novel food before and after exposure of the novel food to

various temperatures and pH levels. Results indicated that miraculin seemed to lose most of its taste-modifying activity at 60°C but stayed stable and active at all the pH levels tested.

The information provided was considered to sufficiently demonstrate stability of the novel food for up to 24 months.

2.5. Specification

The specification parameters for the novel food were provided (Table 6).

Table 6. Specification for DMB

Parameter	Composition
Carbohydrates	70 - 87 g/100 g
Sugars	50 - 75 g/100 g
Fat	0.5 – 3.5 g/100 g
Saturated fatty acids	0.75 – 1.75 g/100 g
Fibre	5 – 10 g/100 g
Protein	4 – 6 g/100 g
Salt	0.01 – 0.06 g/100 g
Miraculin	1.5 – 2.75 g/100 g
Microbiological criteria (CFU/g)	
Total aerobic colony count	≤ $1x10^4$ cfu/g
Bacillus cereus (presumptive)	< 100 cfu/g
Clostridium perfrigens	≤ 50 cfu/g
Yeasts and Moulds	< 500 cfu/g
Staphylococcus coagulase+	<100 cfu/g
E. coli glucuronidase +	<10 cfu/g
Mycotoxins	Absent
Pesticide levels in accordance with Code number 0820990 ('others' in the group of fruit spices) set out in Regulation (EC) No 396/2005.	

Due to the variability reflected in the data between the composition analysis and the specification, further explanation was requested for this variability. The applicant highlighted that this was a whole fruit powder and as such, the novel food would be influenced by external factors such as climate and soil, and the abundance or presence of water, affecting the concentration ranges. This suggests the proposed specification captures the variability in the novel food and is consistent with that applied by other regulators.

The information provided is sufficient for the specification of the novel food and appropriately characterises the novel food seeking authorisation.

2.6. History of Use

Miracle fruit came to the attention of Europeans in the eighteenth century where Auguste Chevalier des Marchais described the fruit of a shrub that had the property to soften what is acidic (Labat, 1725-1730). Chevalier also indicated the fruit was cultivated by natives of Nigeria and Benin. The first thorough description was written by a British surgeon, F.W. Daniell (Daniell, 1852), noting that West Africans consumed it before eating a number of acidic native foods.

Synsepalum dulcificum is currently cultivated in Taiwan, China, the USA, Ecuador, Colombia and Puerto Rico. It is permitted for sale in a number of countries including the US and Japan; safety assessments were not conducted before it was placed on sale.

The history of use does not indicate any further areas for evaluation.

2.7. Proposed Use and Intake

The target population proposed is the adult population. Pregnant and lactating women, and children, are excluded due to there not being enough data to support their safe assessment.

The novel food is to be consumed in food supplements (as defined in The Food Supplements (England) Regulations 2003 and equivalent legislation in the nations of GB) in development of oral formulations such as powders, granules, orally dissolving tablets, lozenges, liquids, and semisolid dosage forms such as gels as an ingredient or as a direct supplement of the dried, powdered fruit. The intention of the supplement is to enhance/modify taste. DMB is proposed at a maximum level of 0.9 g/day (15 mg/kg body weight/day) distributed in 3 servings (0.1 g – 0.3 g per serving) per day before meals/food/beverage.

No other source has been considered for combined intake. There are no precautions or restrictions applied apart from requirements under the GB supplements regulations, for labelling for consumer information on the proposed population for consumption.

The information presented on exposure and proposed uses was considered, and as such it was concluded there were no safety concerns.

2.8. Absorption, Distribution, Metabolism and Excretion (ADME)

The fate of the main constituents of DMB (carbohydrates, ash, protein and micronutrients) are well established in the body. An in vitro study (discussed further below) to analyse the digestibility of miraculin, the glycoprotein that represents 2% of DMB was carried out (Menéndez-Rey, 2018) [Unpublished].

2.9. Nutritional Information

A nutritional analysis of the novel food was provided. It consists of approximately 70%- 90% carbohydrates, 5%-6% protein and 3%-6% ash. The novel food is not intended to replace any existing food. It is intended to enhance the sweetness of certain foods (those that are acidic) as a result of the presence of miraculin in DMB.

It was noted that the miraculin present in the novel food has been identified as a Kunitz trypsin inhibitor, which are known to be stable and resistant to digestion. Two tests were conducted to assess the stability of the proteins in the novel food to digestion: i) a simulated gastric fluid (SGF) pepsin resistance test and ii) simulated intestinal fluid trypsin resistance test. Tests were undertaken on the total protein content of the berry (Menéndez-Rey, 2018) [Unpublished].

The first test explored the stability of DMB when incubated with a range of enzymes found in the digestive tract. The results were visualised using SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) and western blotting using a specific polyclonal antibody raised to miraculin. Reassurance was received that the method was detecting the stability of the miraculin protein and of the appropriateness of the testing strategy applied. The results indicated that miraculin remained stable with a miraculin band appearing on the gel after enzymic treatment with deoxyribonuclease, ribonuclease, lactase, α-amylase, peptidase D and carboxypeptidase M.

The second experiment was conducted to explore the impact of trypsin and chymotrypsin on miraculin. The results of the test were visualised using the specific antibody. Miraculin showed signs of degradation, as seen by a reduction in intensity of the miraculin band on the gel after incubation, with endopeptidases pepsin and trypsin, in line with the natural protein catabolism during human food digestion.

Following concerns raised over whether miraculin has the potential to act as an antinutritional factor by inhibiting the human pancreatic proteases trypsin and chymotrypsin, assays to evaluate the trypsin inhibitor content in 3 different non-consecutive batches of the novel food were conducted.

The test was conducted in line with the protocol described in Welham & Domoney, 2000. Trypsin inhibitors were extracted and quantified. Trypsin inhibitor activity was identified to be between 0.80 and 0.97 TIU (trypsin inhibitor units)/mg on a dry weight basis. This is comparatively lower than for other commonly consumed foods such as roasted peanuts with a trypsin inhibitor activity of 1.28 TIU/mg of sample (Pedrosa, 2021) [Unpublished].

It was concluded that trypsin inhibitor action of the novel food is comparatively low in relation to other commonly consumed food sources suggesting that consumption of this food is unlikely to exert significant inhibitory effects on the human pancreatic proteases' trypsin and chymotrypsin. It was noted that the digestion assays provided confirmatory evidence that miraculin present in the novel food is stable but may be digested by trypsin. It was concluded that the novel food and its components are unlikely to act as an antinutritional factor.

Based on this information, the consumption of the novel food is not expected to be nutritionally disadvantageous for consumers under the proposed conditions of use.

2.10. Toxicological Information

2.10.1. Genotoxicity

In vitro and *in vivo* genotoxicity testing of the novel food was conducted under Good Laboratory Practice (GLP) conditions and utilised the following OECD guidelines: two *in vitro* bacterial reverse mutation tests (OECD TG 471), an *in vitro* mammalian cell micronucleus test (OECD TG 487) and an *in vivo* rat erythrocyte micronucleus test (OECD TG 474). The guidelines used are recommended by the UK Committee on Mutagenicity and is also the basis of guidance on the preparation and submission of an application for authorisation of a novel food in the context of assimilated Regulation (EU) 2015/2283.

The first bacterial reverse mutation test (CERETOX, 2018a) was performed in accordance with OECD TG 471 and in compliance with GLP. The novel food was tested using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537. The assay was conducted in three phases: a preliminary cytotoxicity test at dose levels of 5,000, 1,581, 500, 158, and 50 µg/plate, a mutagenicity main test, and a confirmatory test at dose levels 5,000, 2,500, 1,250, 625 and 312.5 µg/plate in the absence and presence of a metabolic activation system. Sterile water was used as a negative control and sodium azide (without S9) and 2-aminoanthracene (2-AA) (with S9) as positive controls. The overall assessment, together with the biological significance of the results and the lack of a dose-response in all treatments, concluded the novel food was considered not mutagenic.

It was explained that a second assay was performed as it was noted that in the first test, results for one strain TA102 (both with and without S9) in the main and confirmatory test were out of the range of historical control data, as well as issues with contamination leading to confirmatory tests being discarded. This suggested issues with the method impacted the interpretation of the test. As such, the test was done again using a preincubation method.

The second bacterial reverse mutation test (Charles River, 2020b) was performed in accordance with OECD TG 471 and compliant with Good Laboratory Practice (GLP). The novel food was tested using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in the absence and presence of S9-mix at concentrations of up to 5,000 µg/plate. A negative control of 0.5% carboxymethylcellulose sodium salt (CMC) was used. The novel food did not induce a significant dose-related increase in the number of revertant colonies in each of the five strains both in the absence and presence of S9-metabolic activation.

An *in vitro* mammalian cell micronucleus test on mouse lymphoma L5178Y TK+/− 3.7.2C cells was conducted (Charles River, 2020a) in accordance with OECD TG 487 and in compliance with GLP. A 3-hour treatment with S9-mix and 3-hour and 24-hour treatments without S9-mix were performed with concentrations of 31.25, 62.5, 125, 250, 500 and 1,000 µg/mL of the novel food, and a negative control of 0.5% CMC. No cytotoxicity was observed after any treatment, and 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.

The *in vivo* study rat erythrocyte micronucleus test (CERETOX, 2018c) [Unpublished] was conducted in accordance with OECD TG 474 (Mammalian Erythrocyte Micronucleus Test) and in compliance with GLP. The novel food was administered via oral gavage to 5 male Wistar rats at a dose of 2,000 mg/kg bw (body weight), twice, 24 hours apart. A negative control of 0.5% carboxymethylcellulose sodium salt (CMC) was used. There were no statistically significant increases in the number of cells with micronuclei observed compared to the negative control.

Based on the results of the *in vitro* and *in vivo* studies provided and summarised in this document the novel food is not expected to be genotoxic.

2.10.2. Acute toxicology

An acute oral toxicity study in rats was conducted in accordance with OECD TG 425 in a study design following an up-and-down procedure (CERETOX, 2018d) [Unpublished]. Three female Wistar rats were orally administered with the novel food of up to 5,000 mg/kg bw in a solvent carrier. No adverse

effects were observed. The FSA and FSS noted that acute toxicity studies are not pertinent for the safety assessment of novel foods and as such were not taken into account in the assessment.

2.10.3. Sub-chronic toxicity

A 90-day repeated dose toxicity study in male and female Wistar rats was conducted in accordance with OECD TG 408 and in compliance with GLP (CERETOX, 2018b) [Unpublished]. One dose group received the novel food by oral gavage at dose levels of 2,000 mg/kg bw per day. The negative control group (0.5% CMC) was comprised of 10 rats per sex per group. Additional groups of 5 rats/sex/group receiving 0 (control) or 2,000 mg/kg bw per day were included to assess the reversibility or progression of any test item-related changes after a 14-day recovery period.

No systemic clinical signs, behavioural changes, mortality, or effects on food and water intake were observed at either control or treated group. There were no test item-related effects in the treatment group in comparison to the control group. The histological examination recorded some findings in the lung tissue. High incidence of foci of alveolar macrophages or foreign body granulomas were noted in control and treated rats, and this was not considered to be treatment related.

Further clarification was sought on the testing strategy used which explores the effects of one dose of the novel food. It was noted that OECD TG 408 for repeated dose 90-day oral toxicity studies states that if a test at one dose level equivalent to at least 1,000 mg/kg bw/day produces no observed adverse effects and toxicity is not expected based on data from structurally related compounds, a full study with three dose levels may not be necessary.

The selected dose of 2,000 mg/kg bw/day was considered sufficiently higher than the expected dose consumed by humans; hence, it was deemed unnecessary by the applicant to use additional animals for the 90-day study with multiple dose levels. However, the FSA and FSS noted this limited the conclusions that could be reached on dose response relationships.

2.10.4. Human studies

The human data provided was limited to sensory studies examining the acute effects of miraculin on taste (Aguilo Aguayo & Echeverria Cortada, 2018) and (Igarashi et al., 2013; Kurihara & Beidler, 1968; Rodrigues et al., 2016; Tafazoli et al., 2020). While there was no evidence of adverse effects observed from the studies, these were not considered to add to the safety assessment as they were all related to investigating the taste-altering properties of the novel food.

2.11. Allergenicity

Allergenic potential for DMB was considered. The applicant used a testing strategy that is different to that in the guidance for novel food applications (EFSA, 2016). The protein content of the novel food is 5%-6% (w/w) of the novel food's composition. When calculated based on the proposed exposure per serving in the food supplement, this is 18 mg of protein per serving. If it is assumed all of this was miraculin, this would represent 54 mg of miraculin per day being consumed. These levels are higher than those known to cause reactions in sensitive individuals with other food allergies.

A literature review undertaken indicated that there were no reported food allergic reactions to miracle berry or members of the *Sapotaceae* family to which it belongs. It was noted that also in this family of plants are trees from the genus *Palaquium*, known for their white latex production.

Information was also provided on ELISA (enzyme-linked immunosorbent assay) testing using commercial kits for major allergens including walnut, peanut milk, gluten, almond, soya and egg (Valero, 2018). Detection of casein and peanut were identified using the ELISA method. While this provided information on the potential for cross contamination in the production process and the effectiveness of allergen control, this did not further support the allergenicity assessment. Investigation of sources of contamination in the production chain provided reassurance that the potential for cross contamination was being actively managed.

ELISA tests were undertaken to investigate whether miracle berry fruit elicited responses by antibodies generated to Bet v1, a major birch pollen allergen, which show minimal cross reactivity to Bet v homologues. The results indicated that no significant reaction was seen compared to controls. This test did not provide evidence to understand the likelihood of cross reactivity between miracle berry and birch pollen allergens (Bensadon-Naeder, 2023) [Unpublished].

As part of the review of allergenicity, queries were raised on the potential for miraculin to be allergenic given that it is a Kunitz family of trypsin inhibitors which is present as 15%-40% of the total protein in the novel food. These proteins have structures which are known to be stable to thermal processing and *in vitro* digestion. As this can be a marker for allergenicity, this prompted a need for a fuller review.

Analysis of sequence homology between the active protein miraculin and major allergens were undertaken. The amino acid sequence of miraculin was compared to known allergens using a series of databases. The SDAP (Structural Database of Allergenic Proteins) was also used. Cross allergenicity was considered to have been identified if there was 35% or

greater homology for an 80 amino acid long sequence, with at least 6 amino acid identity. Homology was found to proteins in soyabean and potato. This was also supported by review with Allercat pro. Homology was found to Kunitz type trypsin inhibitors in soya bean and potato.

Similarly, searches of the miraculin amino acid sequence in BLAST (Basic Local Alignment Search Tool) revealed that miraculin is a Kunitz trypsin inhibitor with similarity to these types of proteins or their precursors. Analysis was also undertaken using an in-house database of allergens. The validation information for this database was not provided and while consistent with the other analysis of protein homology, the data was considered confirmatory.

The results of the analysis indicate weak homology with Kunitz type trypsin inhibitors in other species. This suggests a very low potential for cross reactivity to soyabean and potato in particular. Cooked potato is a rare cause of food allergy, and soy allergy is not prevalent in the UK (Simpson, et al., 2024).

While some information is available on the digestibility as described in the nutrition section, this could not be directly applied to the allergenicity assessment.

The potential for miraculin to be allergenic could not be ruled out from the data presented. It was noted that there was the potential for *de novo* sensitisation and if an individual was sensitised, the potential for allergic reactions would remain. The proposed use is for 0.9 g of the novel food per day which is lower than other commonly consumed proteins. However, it has not undergone conventional heat processing; hence, any allergenic activity will remain.

3. Discussion

The novel food Dried Miracle Berry is the dried pitted fruit of *Synsepalum dulcificum* with the function of taste modification of acidic fruits.

The target population is the adult population, excluding pregnant and lactating women, and children. The novel food, Dried Miracle Berry, is to be used as an ingredient in oral formulations such as powders, granules, orally dissolving tablets, lozenges, liquids and semisolid dosage forms such as gels or as a supplement of the dried fruit.

It is not intended to replace or substitute any existing food. Whilst it may be a source of nutrients, the main dietary role of this food is for people wishing to mask the acidity and enhance the sweetness of certain foods. As such, its intake is not considered to be nutritionally disadvantageous for the consumer.

The toxicological *in vitro* and *in vivo* studies showed no sub-chronic effects. An indicative value (a top dose NOAEL (no observed adverse effect level) of 2,000 mg/kg bw/day) was provided based on the highest and only dose in the 90-day study. The proposed daily intake of 0.9 g/day corresponds to 13 mg/kg/day for a 70kg adult with a margin of safety of 154, and 39 mg/kg/day for a 23 kg child with a margin of safety of 51. An uncertainty factor of 100 was applied with consideration that the applicant is excluding pregnant and lactating women, and children. The margin of safety was considered acceptable in adults as no adverse events were observed in the clinical trials and sensory analysis published in the literature for the novel food. The applicant's proposed maximum use level of 0.9 g/day was therefore considered to be acceptable for adults. Given the margin of safety was lower in children the applicant's proposal to exclude this group was considered appropriate.

The novel food contains miraculin of below 2.1%, which is the compound responsible for taste enhancement/modification. This compound belongs to the Kunitz-type soybean trypsin inhibitor (STI) family, with antinutritional factor and allergenicity potential. However, assays to evaluate the trypsin inhibitor content suggests this is low in comparison to other commonly consumed foods and unlikely to act as an antinutritional factor. Tests for protein digestibility also indicated low likelihood of allergenicity.

4. Conclusions

The FSA and FSS have undertaken the assessment of Dried Miracle Berry and concluded that the novel food is safe under the proposed conditions of use and does not pose a safety risk to human health. The anticipated intake level and the proposed use in food supplements was not considered to be nutritionally disadvantageous.

These conclusions based on the information in the novel food dossier submitted by the applicant plus the supplementary information, and could not have been reached without the following data claimed as proprietary by the applicant:

- Validation method for miraculin
- Compositional analysis
- Toxicological studies (acute toxicity, genotoxicity and sub-chronic toxicity)
- Human studies
- Allergenicity studies

Abbreviations

Abbreviation	Definition
AA	Aminoanthracene
ACNFP	Advisory Committee on Novel Foods and Processes
ADME	Absorption, Distribution, Metabolism and Excretion
bw	body weight
AOAC	Association of Official Analytical Chemists
CAS	Chemical Abstracts Service
CERETOX	Centre de Recerca en Toxicologia
CFU	Colony forming unit
cGMP	Current Good Manufacturing Practice
dw	dry weight
Dietex	Dietary Exposure
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FDA	Food and Drug Administration
FSA	Food Standards Agency
FSAI	Food Safety Authority Ireland
FSS	Food Standards Scotland
GC-FID	Gas chromatography – flame ionisation detection
GLP	Good Laboratory Practice
HACCP	Hazard Analysis and Critical Control Points
HPLC	High-performance liquid chromatography
ICP	Inductively coupled plasma
KTi	Kunitz trypsin inhibitor
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOQ	Limit of quantification
LOD	Limit of detection
MN	Micronuclei
MOE	Margin of exposure
MS	Mass spectrometry
ND	not determined
NDA	Dietetic Products, Nutrition and Allergies Panel
NF	Novel food
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Co-Operation and Development
PAHs	Polycyclic aromatic hydrocarbons
PCE	Polychromatic erythrocytes
PCR	Polymerase chain reaction
qH-NMR	Quantitative 1H-nuclear magnetic resonance
RH	Relative humidity
RNAse	Ribonuclease

Abbreviation	Definition
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
STI	Soybean trypsin inhibitor
TIU	Trypsin inhibitor unit
UFLC	Ultra-fast liquid chromatography
UPD	up-and-down
USDA	United States Department of Agriculture
w/w	weight per weight

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References

Aguilo Aguayo, I., & 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.

Bensadon-Naeder, L. (2023). *In-Silica analysis of cross allergenicity of the miraculin protein using different predictor tools* [Unpublished report].

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. 73416: 90-Day Oral Toxicity Study of a Lyophilized Novel Food in Wistar Rats with a 14-day Recovery Period* [Unpublished report].

CERETOX (Centre de Recerca en Toxicologia). (2018c). *Study No. IF-74516: genotoxicity study of a novel food: micronucleus test in rats* [Unpublished report].

CERETOX (Centre de Recerca en Toxicologia). (2018d). *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].

Charles River. (2020a). *Dried fruits of* Synsepalum dulcificum *(Miracle Berry Freeze-Dried Powder): A GLP In Vitro Mammalian Cell Micronucleus Test* [Unpublished].

Charles River. (2020b). *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].

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. (2020). *OJ L*, *178/7, 8.6.2020*, 14.

Daniell, W. F. (1852). On the Synsepalum dulcificum, de cand. or, miraculous berry of Western Africa. *Pharmaceutical Journal*, *11*(445), 445–448.

EFSA NDA Panel (EFSA Panel on Nutrition and Novel Foods). (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*, *14*(11), 4594. https://doi.org/10.2903/j.efsa.2016.4594

Igarashi, G., Higuchi, R., Yamazaki, T., Ito, N., Ashida, I., & 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

Kurihara, K., & Beidler, L. M. (1968). Taste-modifying protein from miracle fruit. *Science*, *161*, 1241–1243. https://doi.org/10.1126/science.161.3847.1241

Menéndez-Rey, A. (2018). *No title* [Unpublished report]. Department of Biotechnology and Plant Biology, Higher Tehcnical School of Agronomic Engineering. Food Science and Biosystems.

Pedrosa, M. (2021). *Determination of trypsin inhibitor on dried fruits of Synsepalum dulficicum* [Unpublished report].

Rodrigues, J. F., da Silva Andrade, R., Carvalho Bastos, S., Braganca Coelho, S., & Marques Pinheiro, A. C. (2016). Miracle fruit: an alternative sugar substitute in sour beverages. *Appetite*, *107*, 645–653. https://doi.org/10.1016/j.appet.2016.09.014

Simpson, et al. (2024). Patterns and prevalence of adult food allergy (PAFA). *FSA Research and Evidence*. https://doi.org/10.46756/sci.fsa.ehu454

Tafazoli, S., Vo, T. D., Roberts, A., Rodriguez, C., Viñas, R., Madonna, M. E., Chiang, Y. H., Noronha, J. W., Holguin, J. C., Ryder, J. A., & Perlstein, A. (2020). Corrigendum to "Safety assessment of miraculin using in silico and in vitro digestibility analyses." *Food Chem Toxicol*, *137*, 111136. https://doi.org/10.1016/j.fct.2020.111136

Takai, A. (2013). Secretion of miraculin through the function of a signal peptide conserved in the Kunitz-type soybean trypsin inhibitor family. *FEBS Lett*, *587*(12), 1767–1772. https://doi.org/10.1016/j.febslet.2013.04.026

Theerasilp, S., Hitotsuya, H., Nakajo, S., Nakaya, K., Nakamura, Y., & Kurihara, Y. (1989). Complete amino acid sequence and structure characterization of the taste-modifying protein, miraculin. *Journal of Biological Chemistry*, *264*, 6655–6659. https://doi.org/10.1016/S0021-9258(18)83477-9

Valero, I. M. (2018). *Determination of the allergen content in the freeze-dried powder of miracle berry (Synsepalum dulcificum D.) using ELISA kits* [Unpublished report].

Welham, T., & Domoney, C. (2000). *Plant Science*. *159*, 289–299. https://doi.org/10.1016/S0168-9452(00)00358-7