Meeting

Dried Miracle Berry (DMB®) Additional Information Discussion Paper

Committee Paper for Discussion - ACNFP/163/01

Advisory Committee for Novel Foods and Processes

Application for authorisation of Dried Miracle Berry (DMB®) as a Novel Food.

Application Number - RP1351

Issue

The Committee reviewed this application for the first time at the April 2023 meeting where members requested further information. The Committee is invited to consider the response from the applicant and whether it addresses the request for clarification satisfactorily or if further information is required.

Background

1. In November 2021, the FSA received the submission for Dried Miracle Berry (DMB) from Baïa Food Co (Spain). The novel food consists of pitted fruits of *Synsepalum dulcificum*, dried . It is often consumed for the presence of the substance Miraculin. It serves as a functional food, food supplement taken before consumption of sour foods for palatability (taste modifier). This food has not previously been commercialised in Europe but is eaten in other regions of the world. This application has a positive EFSA opinion (2021).

2. The Committee reviewed this dossier for the first time on 26th April 2023 where further information was sought from the applicant in the following areas: production process, composition, specification, ADME, nutrition, allergenicity and

toxicology.

3. The Committee is asked whether the applicant’s response addresses the outstanding questions from their request for information. To inform the discussion, the FSA requested further information (Annex A) and the applicant’s response (Annex B) are provided. All other supporting data is in Annex C.

Applicant’s response to request for further information

Production Process

4. Members noted the variability within the production process especially in the harvesting and sorting stages. A query was raised on how sorting the fruit in cooled or uncooled conditions would affect the food hazards for the product or whether it was a quality concern.

5. The applicant has summarised their harvesting and sorting practices. This included harvesting being conducted in the morning, visual and secondary sorting, proper packaging and transportation, immediate processing, and refrigeration, when necessary, stating these have been carefully developed to minimize the risk of food hazards and address quality concerns (Annex B). They conclude that a sorting practice, maintenance of low temperatures and ensuring processing is completed within 8 hours of harvest is key for maintaining quality of the product. They also suggest the cool conditions are a component in their management of microbial growth (Annex C: Annex I).

6. Further information was sought on how the risk of fungal disease during harvest and post-harvest stages is controlled. The applicant has outlined a set of measures to control and minimise the risk of fungal disease (Annex B). This includes training of workers in proper management and harvesting techniques (Annex C: Annex II). They also state only compliant batches analysed are released for export, and that 3 batches analysed with the final product lyophilized demonstrates the absence of mycotoxins in those batches and therefore the effectiveness of their systems (Annex C: Annex III).

7. The Committee noted that a detailed HACCP plan was provided but suggested that this would be best summarised in a table for ease of assessment. The applicant provided a summarised table of the HACCP plan (Annex C: Annex IV).

Composition and Specification

8. The Committee noted the variability in the compositional analysis results for the novel food. The applicant was asked to further explain the sources of variability beyond seasonal variability, the impact on the product and how this will be managed.

9. The applicant explains that as the novel food is the whole fruit itself, as such the sources of variation are primarily from the variability in individual plants and the impact of growing conditions. They highlight that difference in rainfall has an impact on composition as it leads to concentration or dilution of the other components in the berry. The nutritional analysis of the last 7 batches produced (Annex B) was provided.

10. They explain that they have developed a standardized specification that accounts for the natural variability observed in the fruit population while still ensuring compliance. They state they select and propagate the best seedling specimen for quality and yield consistency. They have also implemented rigorous quality control which includes regular compositional analysis. Through this they are providing a safe product that meets regulatory requirements and satisfies consumer expectations. They have provided COAs of batches produced in 2022 and 2023 (Annex C: Annex III)

11. The members noted that 4% of the product is polyphenols. Further information was sought on the classes of polyphenol present in the ingredient and whether any of them have antinutritional properties.

12. The applicant has not specifically analysed the polyphenol content of the berries but references information from literature on the berries' composition. Buckmire and Francis (1976) (footnote) quercetin-3-galactoside, kaempferol-3-glucoside, and myricetin-3-galactoside as the primary compounds. Du et al (2013) (footnote) is also sites provide insight into the wider composition of miracle berry.

13. To assure safety the applicant has calculated that 4% polyphenols contained in the freeze-dried powder, this translates to a daily intake of approximately 28mg of polyphenols, which is lower in comparison to amount ingested from fruits rich in polyphenols like blueberries. They conclude that the maximum daily intake of 0.7 grams per person implies that the amount of polyphenols ingested is very low compared with other fruits and would not have a significant negative impact on consumers' health and that these have the added benefit of being rich in antioxidants.

14. The members also noted the protein fraction of the ingredient had been characterised by SDS-PAGE analysis. They queried the basis for ascribing the polypeptides to miraculin and the different mobility polypeptides related to different glycosylated forms of the protein. Questions were also raised on densitometry analysis and whether this provided a strong basis for the estimation of the miraculin content.

15. The applicant has asked that these be removed from the dossier under consideration as these were supplementary tests. Considering the preliminary nature of the findings presented in their report and the speculative nature, the applicant has requested to withdraw this report from the dossier as they recognize that it is not part of the safety assessment and that it does not serve as a method to quantify miraculin in different batches of their product. However, they further explain how they hypothesized that the polypeptides could be related to miraculin (Annex B).

16. The applicant outlined and internally validated a method using 1H-NMR (Proton Nuclear Magnetic Resonance) spectroscopy to quantify miraculin levels. This method serves as their primary approach for quantifying miraculin, and they have consistently applied it to each batch produced. They conclude based on the results of all the analyses conducted on their batches, miraculin content in each batch falls within the range of 1.5% to 2.5%. They state lowest value obtained so far has been 1.7%, while the highest recorded value is 2.5%. Full report in Annex C: Annex V.

17. The higher levels of Chrysene were noted and the applicant was asked to advice whether this has significance for food safety. In response the applicant explains that chrysene and the other PAH’s tested for in 4 batches are very low and fall below the regulatory levels in Commission Regulation (EU) 2023/915. On this basis of the certificates of analysis (COAs) in Annex II, the applicant concludes that this demonstrates low PAHs content which is consistent with a safe product for human consumption.

Absorption, distribution, metabolism and excretion

18. The data in Annex 9 was considered in relation to ADME but it was noted that this information was on miraculin rather than the novel food itself. The applicant was requested to summarise the available data on the ADME of miracle berry or explain the current data set is relevant to the novel foods as a whole.

19. The applicant states that the primary focus of their in vitro ADME study was to investigate the effects and characteristics of the protein extract containing miraculin, which is the key bioactive compound identified in the novel food.

20. ADME data for miracle berries as a whole is not available. They have provided a commentary explaining that the nutritional composition is comparable to berries like strawberries or blueberries. They state that the main constituents of the novel food are carbohydrates, specifically common sugars, including fructose, glucose, and sucrose, and that these sugars follow the normal processes of absorption, distribution, metabolism, and excretion in the human body.

Furthermore, that the low content of lectins in the fruit ensures that the absorption of these sugars remains unaltered. With these factors in consideration, they conclude that the ADME aligns with normal physiological processes.

Nutrition and Allergenicity

21. The Committee noted that miraculin is a highly disulphide bonded protein belonging to the Kunitz-type STI family and as such could have potential to be an antinutritional factor and or implications for the allergenicity of the product.

22. On this basis, they queried if miraculin has the potential to act as an antinutritional factor by inhibiting human pancreatic proteases trypsin and chymotrypsin. The applicant points out that in vitro miraculin digestibility studies have revealed normal protein digestion by action of trypsin. They have also conducted assays to evaluate the trypsin inhibitor content in 3 different non-consecutive batches of the novel food, concluding that trypsin inhibitor content in 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 human pancreatic proteases trypsin and chymotrypsin. A report on these findings is provided (Annex C: Annex VI). The applicant concludes that the novel food and its components are unlikely to act as an antinutritional factor.

23. Details of the allergenicity assessment were explored. Data had been presented on the digestibility of the proteins in the novel food as highly indigestible proteins can be a marker for allergenic proteins. The members queried whether the in vitro digestibility was assessed using a variety of enzymes. They asked how these tests relate to other validated in vitro digestion tests such as those used for allergenicity risk assessment (as indicated in guidance documents).

24. In response the applicant explains the tests had not been conducted for the allergenicity assessment but were considering ADME. To address the allergenicity assessment two tests were conducted a simulated gastric fluid (SGF) pepsin resistance test and (ii) simulated intestinal fluid trypsin resistance test were undertaken on the total protein content of the berry. The protein was digested in these tests suggesting a low likelihood of allergenicity. (Annex B).

25. The members also noted that the SDS-PAGE and immunoblotting was used to provide a read-out of digestibility tests. They queried details of how the tests were performed and under what conditions

26. The applicant explains the process in depth in Annex B, with studies detailed in Annex C: Annex VII, IX and X. The gels were run under reducing conditions. The antibodies were specific to a short sequence of the miraculin protein suggesting that it would pick up both intact protein and protein fragments.

1. In relation to a question on the potential for larger structures to be visualised, they further explain when miraculin is ingested and degraded in the gastrointestinal tract, it is likely that some of the resulting fragments maintain the disulphide bond, giving rise to fragments of higher molecular weight. However, the low number of disulphide bridges and the presence of two of them in the same loop makes the appearance of larger entities unlikely. This informed the interpretation of the digestion assays and the conclusion that miraculin is digested in the gut. The applicant also cites a study by Tafazoli et al (2019) identifying different peptides obtained after pepsin digestion of the miraculin protein (footnote). This group identified by LC-MS/MS a total of 61 peptides after 10 min of digestion and after their comparison against the AllergenOnline database (http://www.allergenonline.org/), they concluded that the resulting fragments do not suggest a risk of allergy to humans, showing a low risk of cross - reactivity with other allergens.
2. The members also queried the bioinformatic analysis of the miracle berry proteins performed to assess for allergenicity. Queries were raised on how the methods used in the bioinformatic analysis compare with the bespoke allergenicity prediction tools generally used in allergenicity risk assessment (e.g. Allercat Pro amongst others), as well as what was the allergenic potential of other proteins in the powder known to participate in fruit crossreactivity syndromes such as Bet v 1 homologues and LTPs.
3. They state that no food allergy caused by species of the Sapotaceae family, to which Synsepalum dulcificum belongs, has been described in the

literature. Despite this, studies have been performed to verify if the total protein content of the pulp and lyophilized skin of Synsepalum dulcificum berries (the NF) presented allergens and/or structural homologues that trigger allergy, results to which are provided in Annex C: Annex XIII.
The applicant explains Annex C: Annex XII shows the results obtained using the AllerCatPro and SDAP allergenicity prediction computational tools, in addition to the applicants prediction method, concluding none of them identified relevant cross-reactive allergens.
To investigate on the allergen Bet v 1, an ELISA assay was carried out (and/or structural homologues) in the novel food, results to which are provided in Annex C: Annex XIV suggesting the absence of this type of allergen.

4. Members noted in the allergenicity section that some signals were coming through for peanut and casein and that this was thought to be a result of cross contact in the supply chain. They sought further consideration to report on the likely sources of cross contact and how this is managed. The applicant highlights the points within the supply chain where there is direct contact with the product, explaining the specifications sheet for the pulp (Annex C: Annex X) shows chances of contamination are low, and that Annex XI contains a COA stating absence of cross-contamination, with the novel food free from cross contamination with one of the major allergens tested for.
5. They also explored the potential for cross reactivity between the novel food and cite Menéndez-Rey et al (2022) (footnote) where cross reactivity between miracle berry and peanut was not seen.

Toxicological information

27. The members reviewed the toxicology information. They requested justification for the rationale of the dosing and explanation how a conclusion was drawn with such a narrow safety margin. They clarify their rationale on the single limit dose for the 90 day oral toxicity study and that this was based on several factors. Historical lack of signs of toxicity within the communities consuming this food as well as the primary acute toxicity testing of the dried fruits showed no adverse effects, even at a high dose of 5000 mg/kg in rats, indicating that the lowest lethal dose would be above that level.

They highlight OECD test guideline 408 for repeated dose 90-day oral toxicity studies states that if a test at one dose level equivalent to at least 1000 mg/kg body weight/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 2000 mg/kg/day was considered sufficiently higher than the expected dose consumed by humans, hence it was deemed unnecessary to use additional animals for the 90-day study with multiple dose levels. The applicant concludes this approach provided evidence that the novel food is safe at the proposed intake level.

Committee Action Required

- The Committee is asked whether the response from the applicant is sufficient to clarify the concerns discussed at the last meeting.
- If not, the Committee is asked to indicate what further data is required and the feedback that should be given to the applicant.

ACNFP Secretariat

November 2023

Annexes

Annex A - Request for further information

Annex B - The applicants response

Annex C – Supporting documents

1. Buckmire, R. E., & Francis, F. J. (1976). Anthocyanins and flavonols of miracle fruit, Synsepalum dulcificum, Schum. Journal of Food Science, 41(6), 1363-1365

2. Du, L., Shen, Y., Zhang, X., Prinyawiwatkul, W., & Xu, Z. (2014). Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts. Food chemistry, 153, 279-284.

3. Tafazoli, S., Vo, T. D., Roberts, A., Rodriguez, C., Viñas, R., Madonna, M. E., ... & Perlstein, A. (2019). Safety assessment of miraculin using in silico and in vitro digestibility analyses. Food and Chemical Toxicology, 133, 110762.

4. Menéndez-Rey, A., Jerez-Arroyo, F., Alegría-Aravena, N., Quiroz-Troncoso, J., González-Martos, R.,

   Sánchez-Díez, M., ... & Ramirez-Castillejo, C. (2022). Discarded 21 cross-allergy between miracle berry (Synsepalum dulcificum) and peanut. International Journal of Food Science & Technology.

5. Buckmire, R. E., & Francis, F. J. (1976). Anthocyanins and flavonols of miracle fruit, Synsepalum dulcificum, Schum. Journal of Food Science, 41(6), 1363-1365

6. Du, L., Shen, Y., Zhang, X., Prinyawiwatkul, W., & Xu, Z. (2014). Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts. Food chemistry, 153, 279-284.

7. Tafazoli, S., Vo, T. D., Roberts, A., Rodriguez, C., Viñas, R., Madonna, M. E., ... & Perlstein, A. (2019). Safety assessment of miraculin using in silico and in vitro digestibility analyses. Food and Chemical Toxicology, 133, 110762.

8. Menéndez-Rey, A., Jerez-Arroyo, F., Alegría-Aravena, N., Quiroz-Troncoso, J., González-Martos, R.,

   Sánchez-Díez, M., ... & Ramirez-Castillejo, C. (2022). Discarded 21 cross-allergy between miracle berry (Synsepalum dulcificum) and peanut. International Journal of Food Science & Technology.

9. Buckmire, R. E., & Francis, F. J. (1976). Anthocyanins and flavonols of miracle fruit, Synsepalum dulcificum, Schum. Journal of Food Science, 41(6), 1363-1365

10. Du, L., Shen, Y., Zhang, X., Prinyawiwatkul, W., & Xu, Z. (2014). Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts. Food chemistry, 153, 279-284.

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12. Menéndez-Rey, A., Jerez-Arroyo, F., Alegría-Aravena, N., Quiroz-Troncoso, J., González-Martos, R.,

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13. Buckmire, R. E., & Francis, F. J. (1976). Anthocyanins and flavonols of miracle fruit, Synsepalum dulcificum, Schum. Journal of Food Science, 41(6), 1363-1365

14. Du, L., Shen, Y., Zhang, X., Prinyawiwatkul, W., & Xu, Z. (2014). Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts. Food chemistry, 153, 279-284.

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16. Menéndez-Rey, A., Jerez-Arroyo, F., Alegría-Aravena, N., Quiroz-Troncoso, J., González-Martos, R.,

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17. Buckmire, R. E., & Francis, F. J. (1976). Anthocyanins and flavonols of miracle fruit, Synsepalum dulcificum, Schum. Journal of Food Science, 41(6), 1363-1365

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19. Tafazoli, S., Vo, T. D., Roberts, A., Rodriguez, C., Viñas, R., Madonna, M. E., ... & Perlstein, A. (2019). Safety assessment of miraculin using in silico and in vitro digestibility analyses. Food and Chemical Toxicology, 133, 110762.

20. Menéndez-Rey, A., Jerez-Arroyo, F., Alegría-Aravena, N., Quiroz-Troncoso, J., González-Martos, R.,

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