Consultation

Consideration by the ACNFP of Bambara Groundnut (Vigna subterranea (L.) verdc.) as a traditional food from a third country

Background

At the 171st meeting of the Advisory Committee on Novel Foods and Processes (ACNFP), a notification for Bambara groundnut (*Vigna subterranea* (L.) verdc.) was considered.

The applicant is requesting authorisation within Great Britain (GB) market for the product as dried whole seeds (hulled and deshelled) and flour processed from cooked dried whole seeds, with the general population as the target population.

The Committee’s discussion

The advice of the Committee to the Food Standards Agency (FSA) and Food Standards Scotland (FSS) is summarised below. Please note the Committee did not consider any potential health benefits from consuming the food as the focus of the traditional food assessment is to ensure the food is safe, not misleading, and not putting consumers at a nutritional disadvantage.

Identity of the traditional food

Bambara groundnut is a legume crop native to Africa that grows underground. It is of the species *Vigna subterranea* (L.) verdc. The crop is hulled and the traditional food consists of the cream-coloured mature seed. Genetically, Bambara groundnuts share the same genus (*Vigna*) to mung beans and cowpea.

Members commented on the lack of a formal variety or strain that has been cultivated or identified and then specified in the dossier. Genetic and phenotypic

variation was not addressed by the applicant and was therefore unknown. Any impact on composition this may have, such as macronutrient or antinutrient levels, could not be assessed.

The applicant had indicated that cultivation is widespread across northeast, west central and western regions of Africa and elsewhere in Asia, Australasia, Caribbean and countries of the Indian Ocean.

The applicant had indicated Ghana in Africa as the origin of the traditional food for the purpose of this application. However, Members raised that geographical scope of cultivation for acceptance of the notification for Bambara groundnut was not clear. The Committee commented that heavy metals and other contaminants in the food would vary according to location of cultivation. The impact of geographical origin on the variation in composition of the traditional food had not been addressed in the notification or in selecting samples for compositional analysis, as described below.

The Committee emphasised the scope for these types of authorisations would be important as it could indicate the need to consider additional hazards for review.

Production Process

The Committee raised concerns that information had not been provided to demonstrate that there are adequate hygiene controls in place for the whole process. Quality assurance and food safety management had not been applied to the full production process from cultivation to end product. Information was provided on quality control and food safety management related to the final processing location only.

As such, it was difficult to reach conclusions on the effectiveness of management during cultivation and harvesting.

Members agreed that the HACCP information did not provide sufficient assurance that microbial safety, in particular the potential for mould growth and mycotoxin production, were being managed appropriately nor were the controls adequate. Information would be needed on whether best practice in mycotoxin management were being applied, in addition to end of production testing, to ensure appropriate controls were in place and mitigating any risks present. Furthermore, long and variable storage periods and transport conditions raised further concerns over controlling the hygiene and microbial safety of the food.

Queries were raised on some of the controls in place. Specifically, the Committee noted that information had been provided on use of cleaning agents but not on the effectiveness of these measures, particularly where two forms of wash could be used. Therefore, the potential additional risks from use of these agents and the potential for risks from chemical residues could not be established without supporting data.

In conclusion, the production process did not provide assurance that safety hazards were being sufficiently managed. Significant points on microbial safety were raised and would need to be addressed to assure safety.

Compositional data

The Committee commented that the compositional data did not provide reliable information on the expected variability of the traditional food. This was due to inconsistency in the batch analyses and a lack of information on each batch. It was unclear the origin of the samples used to generate the data. Information on harvest year, geographical location, controls and storage were missing for individual batches to support their value in review.

It was noted by the Committee that batches of the dried seeds and flour had not been analysed across all parameters per independent batch as a complete profile. Instead, separate batches had been analysed for individual parameters. Therefore, the data could not be compared between the two products to understand the impact on control measures. This raised questions on whether the sources of variability in the novel foods production had been appropriately explored to support safety of the product.

Variability in the levels of mycotoxins were noted by the Committee. Notably, the levels of Aflatoxins in the flour product. The Aflatoxin B1 level in one batch (2.06µg) was above the legal limit of 2 µg stipulated in assimilated Commission Regulation (EC) No 1881/2006 setting the maximum levels for aflatoxins in related food categories (processed products of groundnuts). The sum of aflatoxins (B1, B2, G1 and G2) in the same batch (3.43 µg) was also close to the legal limit of 4 µg.

The applicant did not justify why mycotoxin levels were significantly higher in the processed flour product compared to the starting dried seeds raw material. This is a different trend to that normally seen for this type of product following physical processing. This finding did not correlate with the information provided on food safety management and the production process. As such, conclusions on safety in

relation to the production of and presence of mycotoxins was difficult to reach.

Members commented that from the analysis presented a trypsin inhibitor was present in the flour product. However, as this was post cooking, this raised questions on whether the cooking step was effectively managing this hazard. Intended consumer uses for the flour were not well defined and as such whether this presented a risk was difficult to establish.

In conclusion, the batch analysis did not provide full assurance on the safety of each batch of the traditional food independently produced. Of particular concern was microbial safety and mycotoxin levels. Compositional consistency had not been demonstrated nor the expected variability for the whole food.

Stability

The Committee noted that stability studies had been conducted on the end products – the hulled dried whole seeds and flour. However, stability during varying storage and transport duration and conditions was not known. This raised further concerns for microbial stability, such as, the potential for mycotoxin production.

Specification

The applicant specified Bambara groundnut in two forms: dried whole seeds and dried seeds that are cooked and milled into a flour.

The Committee highlighted that the yeast and mould counts and total plate counts were set too high for both forms. Given the long storage periods for the food prior to shipping, the specifications raised significant concerns and demonstrated a lack of microbial control. The risk of mycotoxin growth was identified as high.

The protein content was noted to be consistent in the compositional analyses but the proposed specifications for this parameter was not supported by this finding.

In conclusion, the specifications were not deemed robust on the basis of acceptable food hygiene and would need to be amended to support the use of effective controls to minimise microbial growth.

Nutrition

The risk of unknown and undesirable phytotoxins and antinutrients in the whole food was raised by the Committee.

Nutritional disadvantage was not well evaluated by the applicant in the intended role in the GB diet. Potential displacement of other foods with a higher nutritional value was noted by Members.

The Committee commented that protein digestibility was not determined by the applicant. On this basis, nutritional disadvantage could not be fully assessed.

Allergenicity

The potential for a risk to legume allergic consumers was highlighted by the applicant. However, the data provided was lacking and further interpretation and evaluation on the potential risk highlighted would have been useful.

The potential for cross reactivity between the traditional food and other legumes was evaluated by the Committee. Since Bambara is a legume, and like the important legume allergen, peanut, it belongs to the subfamily of the Fabaceae (or Leguminoseae) which are known as the Faboideae or Papilionoideae. Thus, there is the potential for the novel food to pose a risk of reaction to individuals with allergies to legumes such as peanut as a consequence of cross-reactive IgE. Cross-reactive allergies between peanut and lupin have been demonstrated (Moneret-Vautrin et al., 1999). However, Bambara, like mung bean, belongs to the clade Hologalegina which is only distantly related to both the clade Dalbergieae, to which peanut (Arachis hypogea) belongs, and the Genista, to which lupin (Lupinus angustifolius) belongs (Doyle, 2001).

Similarity between Bambara and other legume allergens was considered by the Committee. However, the data to support bioinformatics analysis based on protein sequence is limited for this species. The data does not include seed storage proteins which would be most relevant for an analysis of potential allergenicity for this traditional food.

Proteomic analysis has identified seed proteins corresponding to the 7S seed storage globulins (Okpuzor et al., 2010) which had significant sequence similarity to the related proteins found in other species of Vicia, including Vicia radiata (mung bean) and Vigna unguiculata (black eyed pea, cow pea). Considering these data, taken with the phylogenetic relationships with related legumes, it is likely that levels of sequence identity between Bambara seed proteins will be similar to that seen for mung bean and cowpea for which more sequence data are

available.

Analysis of the data for mung bean suggests the sequence similarity was greatest between mung bean and soybean 7S seed storage globulin (also known as the allergen Gly m 5) but significantly lower with the peanut allergen 7S seed storage globulin Ara h 1 (~50%). Sequence analysis of the aligned sequences between mung bean 7S globulin and soybean Gly m 5 showed regions where the sequences are very similar, although sequence identities are interspersed with sequence similarities which would likely disrupt IgE-epitopes.

The applicant demonstrated a lack of presence of a Bet v 1 homologue in the samples using immunoassay. This indicates that, although there is likely to be a PR10 protein expressed in the seed, it is at either a very low level or is sufficiently different to Bet v 1 to not cross-react. In either eventuality, it indicates that this is unlikely to present an issue for individuals with inhalant allergies to bet v 1.

These in silico data indicate that the mung bean proteins have the potential to be cross-reactive with IgE from patients with allergies to legumes such as peanut, soybean. However, there is uncertainty over what levels of homology is a prerequisite for cross-reactive allergies, although much higher levels of homology are associated with clinical cross reactivity, such as walnut and pecan (Brough et al., 2020, Nesbit et al., 2020) with the 7S seed storage globulin allergens for walnut (Jug r 1) and pecan (Car i 1) having sequence identities of 92.71% (Mills et al., 2024).

Human data on clinical reactivity to Bambara was limited. There is one poor quality study using human serum samples from poorly characterised food allergic patients which purports to show IgE cross-reactivity (Astuti et al., 2016). Another study employed skin prick testing (SPT) as part of a study to develop a skin testing reagent. However, clinical characterisation of the patients is very poor (one is described as having an allergy to cold) and states none of the patients included were allergic to Bambara. IgE was determined by an in-house ELISA, immunoblotting and SPT. The SPT results showed all individuals tested had reacted to the SPT reagent. However, only 5 had reported reactions to peanut and tree nuts (Chalid et al., 2015).

Based on the phylogenetic relationship of Bambara to other legume foods such as peanut and soybean, the traditional food presents a low risk of causing clinical reactions in individuals with allergies to other legumes, such as peanuts. Bambara is therefore very unlikely to cause reactions in individuals with inhalant allergies to PR10 homologues found in birch, alder and hazel pollen.

Depending on exposure (levels of consumption and types of food products in which Bambara is included) over time it is possible that Bambara may emerge as a new allergen in a manner similar to pea isolate (Abi-Melhem and Hassoun, 2023, Lavine and Ben-Shoshan, 2019). Factors such as poorer digestibility, level of use and type of food categories, such as those used as dairy and meat replacers, will likely increase the risk of new allergies developing.

Experience of continued use

The Committee argued whether safe history of use had been demonstrated. It was raised that the food appeared to be consumed at a domestic level and often in place of other foods in times of scarcity. The Committee felt that the data provided did not provide certainty that this was a staple food that has been consumed widely over a period of extended use. It was additionally noted that history of use was provided on different regions in Africa rather than a focus on safe use over an extended period of time within specified countries and populations. As such, a safe history of use was not fully understood.

This was complicated further by the lack of clarity on whether there are defined varieties of Bambara or whether its phenotypic characteristics are variable.

Proposed conditions of use for the UK market

The Committee noted that the product is referred to as a nut which could mislead consumers particularly for those with nut allergies. As described above, Bambara is more similar to other legumes that are also grown in the ground.

The Committee discussed the scope of the acceptance criteria for an authorisation. The applicant had not clearly defined the geographical locations of harvest, cultivation and processing should this be accepted for the GB market. Additionally, the intended uses, use categories and forms of use were loosely defined. This made comparison to the traditional use more difficult. The Committee highlighted that while under the regulations the novel food could only be sold in the food categories as authorised and not as an ingredient in other food categories, the dried seeds and flour could be used in many different ways by consumers.

The Committee raised that cooking requirements for either the seed or the flour was not made clear. This raised safety concerns regarding the expected levels of antinutrients in the products as consumed. The Committee commented on

specific examples, such as levels of lectins and trypsin inhibitor, and the risk of GI symptoms or toxic effects.

Conclusion

The Committee identified several areas of concern and raised a lack of reassurance that hazards were being adequately controlled. Human health risks were identified by the Committee and, in their opinion, anticipate food safety risks to the GB population. The Committee's view was that various sections of the application, namely identity, production process, composition and proposed uses would need to be explored further to provide assurance on safe use.

The main areas of uncertainty were microbiological safety, mycotoxin levels, antinutrient levels and protein digestibility. Compositional variability was poorly understood for the dried seeds and flour across all parameters. The Committee were of the opinion that food safety management, controls and quality assurance as described were insufficient. The hazards arising from the product in storage and transport was raised as a safety concern.

Allergy to legumes is a known issue in the wider UK population. A low risk of clinically relevant reactions in individuals with allergies to other legumes, such as peanuts was concluded. However, the risk of de novo sensitisation remains for this type of product.

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