ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR TOUCHI EXTRACT DERIVED FROM FERMENTATION OF SOYA BEAN BY *Aspergillus oryzae* AS A FOOD INGREDIENT

Applicant: **CBC Co Ltd**

Responsible Person: **Mr Shinya Miyairi**

EC Classification: **2.1**

Introduction

1. An application was submitted by CBC Co. Ltd for the authorisation of Touchi extract derived from the fermentation of black bean (*Glycine max*) by *Aspergillus oryzae* as a novel food ingredient.
2. Touchi extract is a protein-rich powder obtained by water extraction of small soybeans fermented with *Aspergillus oryzae* and contains an alpha-glucosidase inhibitor. It is intended to be consumed by people who wish to slow the breakdown of carbohydrate following a meal.
3. This application for the authorisation of Touchi extract was prepared pursuant to Commission Recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. Touchi extract has been classified as a complex novel food from a non-GM source, where the source of the novel food has a history of consumption in the EU (class 2.1).

I. Specification of the novel food

Application dossier, pp 4-12

4. The raw material used to prepare the novel ingredient (NI) is a variety of soybean (*Glycine max*) which has been extensively used in the Sichuan province of China for centuries and is also known as the small yellow bean. Fermentation is performed using *Aspergillus oryzae* which has an established use for many years in the production of soy sauce, miso and sake.

5. The NI is a light brown powder and the applicant has provided the following proposed specification which includes both nutrient and purity limits:

Specification for Touchi Extract	
Parameter	**Specification**
Characteristics	
Appearance	Light brown powder
Taste	'Pleasant"
Fat	Max. 1%
Protein	Min. 55%
Water	Max. 7%
α-Glucosidase inhibitor activity	IC_{50} min. 0.025
Contaminants	
Arsenic	Max. 10 µg/kg
Aflatoxins	Max. 5 µg/kg
3-MCPD	Max. 50 µg/kg
Total heavy metals (expressed as lead)	Max. 20 µg/kg
Microbiological Requirements	
Total bacteria count	≤ 1000 cfu/g
Total mould and yeast count	≤ 300 cfu/g
Escherichia coli	Negative /g

6. The applicant states that no pesticides are used in the production of the NI. Results of a pesticide residue screen show none were present at or above the limits of detection.

7. The applicant has provided an analysis of potentially toxic inherent constituents, external and process contaminants, for three non-consecutive batches of the NI which are representative of the commercial product to be marketed in the EU.

8. Heavy metal analysis show levels of lead and cadmium are within acceptable ranges (0.2 µg/kg limit for lead in cereals, legumes and pulses and for cadmium in soybean, according to EU legislation) and levels for arsenic and mercury are also below the limit of detection.

9. At the proposed level of intake for the NI, the levels of dioxins and dioxin-like PCB's analysed in three batches are considered to fall within acceptable ranges. Levels of Polycyclic Aromatic Hydrocarbons (PAH) are also below detection limits.

10. The applicant's mycotoxin screen indicated that none of the batches of the NI contained aflatoxins B_1, B_2, G_1 and G_2 at the limit of detection (<0.2 µg/kg). The applicant states that as far as it is aware *Aspergillus oryzae* is not a source of ochratoxin A and this statement was supported by an expert at an accredited independent testing laboratory.

11. The level of 3-monochloropropane-1,2-diol (3-MCPD) was determined for three batches of the NI. The levels of 3-MCPD were found to be <30 µg/kg for the three

batches tested and below the EU regulatory requirements (up to 50 µg/kg in dry matter). The applicant highlights that the maximum intake level of up to 4.5 g/day proposed for the NI ensures this product will not contribute significantly to the dietary intake of 3-MCPD.

12. The applicant notes that one portion of a dish using traditional black bean sauce would generally contain 15 g of fermented black beans, which on extraction, is equivalent to 4.5 g of the NI. During extraction of fermented black beans, the water soluble fraction is removed from the insoluble part to give a product that is high in protein but low in fat. The applicant states that, as no chemical modification is involved in the extraction of the water-soluble fraction of fermented black bean, the nature of the nutrients remains identical to the traditional fermented counterparts and therefore the NI is essentially a concentrated form of the protein fraction of fermented black beans. The applicant has provided a summary of the fat and protein content of 3 batches of the NI in Table I.C.2.1-1 of the application dossier.

13. Fermented black beans exhibit alpha-glucosidase inhibitory action and on extraction this activity is retained. The IC_{50} for inhibition of alpha-glucosidase ranged from 0.05 to 0.55 mg/mL in three batches of the NI. The NI was also screened for inhibition of other enzymes but only alpha-glucosidase inhibitory action was observed.

14. The NI is intended to be used as a powder or incorporated into formulations for presentation to the consumer as a food supplement. In order to confirm the stability of the NI the product was monitored as bulk powder, tablet form and tea formulation. The NI was observed to be stable for over 36 months at room temperature based on these studies. In addition, results of accelerated tests at 55°C and tests as a 10% (w/w) solution in water were provided

***Discussion**: The Committee accepted the applicant's proposed specification for the NI and was satisfied with the stability tests. The Committee noted that the wide range of IC_{50} values reported for the NI may affect the efficacy of different batches of the product but not their safety. (Please also refer to paragraph 27 below.)*

II. Effect of the production process applied to the novel food

Application dossier, pp 13-17

15. The applicant provides a basic overview of the fermentation process in the application dossier, noting that this is consistent with the conditions used in the production of black bean sauce. Small soybeans are washed and screened for foreign material, then steamed and fermented in an aerobic environment using the fungus *Aspergillus oryzae*. The resulting fermented black bean (the fermentation process blackens the beans) is washed, dried, screened for any foreign materials and packaged under vacuum until it is used in the extraction stage.

16. Fermentation using the fungus *Aspergillus oryzae* is a well established procedure used in the production of soy sauce, sake and miso. The applicant is of the view that the exact fermentation conditions that lead to a high quality product without risk of toxic by-product formation have been established over years of industrial experience thereby ensuring the safety of the NI.

17. The fermented beans are milled and then suspended in boiling water. The aqueous phase is separated from the insoluble fraction following a series of purification steps before being concentrated and spray dried to create the final product, which is a pale brown powder. The applicant states that the process is typical of the industry and follows conventional extraction procedures.

18. The applicant contends that there are no potential hazards associated with the processes employed in the production of the NI and no hazardous materials are formed under proper, regulated manufacturing conditions using *Aspergillus oryzae*. The procedures involved in the manufacture of the NI follow the principles of HACCP and GMP. The process and intermediate products are monitored routinely to ensure that the NI meets the specification. If the final product does not meet the required specification, it is discarded.

19. The manufacture of the NI complies with principles laid out in Regulation (EC) No 852/2004 on the hygiene of foodstuffs at all stages of the production process. The preparation of the NI in powder form lowers the amount of moisture to <15% thereby reducing the potential for microbial growth and increasing the shelf life of the product.

20. In response to the Committee's request for further information on the similarity of the heat-treated NI to black bean sauce, the applicant provided examples of recipes involving the use of fermented black beans to make a sauce. The applicant notes that although the production of the NI involves 2 separate heat treatments, the second treatment is the sterilisation step and is a controlled treatment performed over a short period of time (135°C, 15 seconds). By comparison, the one heat treatment involved in preparing fermented black bean sauce involves higher temperatures and less controlled conditions. Nevertheless, the applicant was of the view that any general effects of heat treatment on the protein components will be consistent between the sauce and the extract.

21. In response to a further request from the Committee the applicant provided the results of HPLC analysis to demonstrate the effect of fermentation and hot water treatment on the protein content of the product. The chromatograms obtained for the NI and fermented black bean paste were very similar and exhibited peaks consistent with low molecular weight peptides and amino acids. The applicant concluded that these results confirm there are no significant differences in composition between the two products.

22. The Committee also requested further details of the fermentation conditions used in the manufacture of the black beans and clarification as to whether the black bean fermented product is the same as the one used to create traditional black bean sauce. The applicant states that fermentation is carried out in an aerobic environment at 30°C over 7 days using the fungus *Aspergillus oryzae*. The fermentation is stopped by cooling before the mixture is washed with water and dried in a dedicated drying room. The applicant confirms that the fermentation process used in the production of fermented black bean sauce and paste is identical to that used in the manufacture of the NI.

***Discussion**: The Committee was satisfied that the production process for the NI did not give cause for concern, compared with the process for traditional black bean sauce. Members also noted that because the raw material in the fermentation process can only be slowly utilised by the fungus, the culture is unlikely to encounter conditions that could result in secondary metabolites. The Committee was content that the additional HPLC chromatograms provided by the applicant indicated that the peptide and protein compositions of the NI and black bean paste were similar.*

III. History of the organism used as a source of the novel food

Application dossier, pp 18-21

23. The raw material used to prepare fermented black bean is a non-GM variety of soybean (*Glycine max*) which has been widely used in the Sichuan province of China for centuries where it is known as the small yellow bean (the fermentation process blackens the beans). The plant grows annually and is native to East Asia, and as it produces much smaller beans than those usually cultivated it is viewed to be a niche variety and as such has not been subject to genetic modification.

24. The soybeans used by the applicant are cultivated by contract farmers in the Sichuan province. There are no agro-chemicals used on the crop and there are no potential sources of pollution in the vicinity of the contract farms. During harvesting, countermeasures are employed to reduce possible contamination with foreign materials (e.g. stone, chips and husk) and the storage facilities are maintained to appropriate hygiene standards which are fully documented to ensure traceability.

25. The fermentation of the small soybean with *Aspergillus oryzae* has been used for hundreds of years in the production of soy sauce, miso and sake. *Aspergillus oryzae* is a member of the *Aspergillus flavus* group and has undergone extensive selection over the years in order to produce the current strains that are adapted for use in fermentation processes.

26. The fermented black bean product from which the NI is obtained has a long history of safe use in China and more recently in Europe as a component of many Chinese dishes.

***Discussion**: The Committee accepted the applicant's view that small yellow soybean and Aspergillus oryzae have a long history of use throughout the world in the production of products such as soy sauce, miso and sake.*

IX. Anticipated intake/extent of use of the novel food

Application dossier, pp 22-26

27. The applicant intends that their NI will be used during a meal in order to hinder the digestion of carbohydrates in the small intestine.. Any health claims that are attributed to the consumption of the NI are not considered as part of this application. Any health or nutrition claims that may be made for this ingredient would be subject to a separate authorisation procedure under regulation (EC) 1924/2006

28. The NI will be marketed in food supplement-type products at levels that would not exceed 4.5 g per daily serving/dose. The form of the supplement may vary (e.g. tablet or tea/soup-style) but the applicant has stated that all products will be clearly marked with an indication of dose of the NI to indicate that the NI should only be consumed with food. The applicant anticipates that consumers will be familiar with this type of food supplement form and will follow the directions for use. In further information submitted to the Committee, the applicant advised that all powdered tea/soup formulations are specifically designed for consumption by mixing with hot water only and are not intended for addition to other foods.

29. The applicant considers that, as the NI will only be available in clearly marked food supplement products, presentation to the consumer will be controlled to provide a dose of the NI that would not exceed 4.5 g per serving/dose per day.

30. The applicant provides intake data from the UK provided as part of the “Concise European Food Consumption Database” to estimate the worst case scenario whereby adult consumers (16 to 64 years) of teas and soups consume these types of products as part of their conventional food intake. Typical UK food portion sizes for a “mug of tea” of 240 g/serving and “cup-a-soup” type products of 215 g/serving have been used to convert the recorded consumption of these foods to servings/day, which are then translated into NI consumption based on the maximum proposed use level of 4.5 g/serving.

31. The mean estimated total intake of the NI consumed as conventional “cup-a-soup” type product and as a tea (assuming all consumption in this category is in the form of tea) are less than 4.5 g and 13.5 g respectively (equivalent to <1 and approximately 3 servings, respectively). The highest level estimated consumption (97.5^{th} percentile of users) of the NI is 4.5 g and 36 g, respectively (equivalent to 1 or 8 servings approximately).

32. The applicant considers that it is unlikely that consumers will be high level consumers of both “vegetable soups” and “tea, coffee and cocoa” and in this assessment it is assumed that the highest level consumers would be high consumer of one category and only average consumers for the other. For high level consumer of “soups” and average for “tea” and vice versa result in estimated total exposure to the NI of 18 g and 40.5 g, respectively. In addition, the applicant points out that the NI is intended only to be consumed with food, which would eliminate any intake associated with the casual consumption of tea or soup between meals.

33. A NOAEL of 2,500 mg/kg bodyweight/day of NI (see paragraph 49 below) equates to 150 g/day of the NI for a typical 60 kg adult. The average intakes of approximately 3 servings of “tea, coffee and cocoa” and less than half a serving for “vegetable soups” combined lead to a maximum intake of less than 4 servings or less than 18 g/day of the NI and an 8 fold safety factor. The highest estimated consumption of 40.5 g/day gives a 3.7–fold safety factor.

***Discussion**: The Committee considered that the consumption of the NI at the proposed levels of incorporation did not raise any specific safety concerns, based on the safety data provided and the history of consumption of traditional fermented black beans (see below). The Committee sought clarification from the applicant on the meaning of the term ‘nutritional support’ which is used in the company’s documentation and accepted that this term was used only to convey that the anticipated consumption of the NI is during a meal rather than on its own.*

X. Information from previous human exposure to the novel food or its source

Application dossier, pp 27-28

34. Fermented black beans have been widely consumed as a traditional seasoning in China for the last 1000 years and in Europe they have been consumed in Chinese dishes containing “black bean sauce” or "black bean paste” as a seasoning typically at levels of around 15 g per serving.

35. In accordance with the terms of Directive 2003/89/EC all products containing the NI will be clearly labelled as made from soya or soybeans. The applicant is of the view that as the NI will only be used in food supplements, the presence and origin of the ingredient will therefore be clearly apparent to the consumer.

36. The applicant states that proteins in soya bean, particularly the ‘storage proteins’ vicillin and legumin, are thought to be responsible for the allergic response of certain individuals to soya-containing products. During the fermentation process, degradation may occur to form fragments that do not exhibit the same allergenic response in susceptible individuals. This does not alter the requirement to label products containing the NI in accordance with Directive 2003/89/EC, unless the applicant applies for an exemption under that Directive.

37. The NI is currently approved as a Food for Specific Health Use (FOSHU) in Japan where it is sold through its direct mail channel in the same supplement-style products proposed for the EU. In post-marketing monitoring conducted by the manufacturer in 2005, no adverse effects were reported or have been reported to date. In response to a request from the Committee regarding the reporting of adverse effects if the product is marketed in the EU, the applicant has advised that post-market monitoring will be conducted via the manufacturer's helpline to ensure any complaints of adverse effects can be communicated to the applicant and to the ingredient manufacturer.

***Discussion**: The Committee was satisfied that yellow soybean products have been consumed as a traditional seasoning in China for many years and that there were no concerns regarding general safety of the beans. The Committee noted that the applicant will provide a mechanism for EU consumers to report any adverse effects from consuming the NI. The Committee requested that the results of any post-market monitoring should be reported after an interval of 18 months.*

XI. Nutritional information on the novel food

Application dossier, pp 29-32

38. In terms of 'nutritional safety' the applicant considers that the NI is equivalent to fermented black beans and fermented black bean paste which have been on the market in the EU for many years.

39. One portion of black bean sauce in a dish would generally contain 15 g of fermented black beans and on extraction; 15 g of fermented black bean sauce corresponds to 4.5 g of the NI. The applicant provides a comparison of nutrient profiles for 15 g fermented black beans and 4.5 g of NI in Table XI.A-1 of the dossier. The applicant states that, whilst the relative amount of each nutrient changes during processing because the water-insoluble fraction is removed, no chemical modification is involved and hence the nutrients that are present in 4.5 g of the extract are also present in the same amounts in 15 g of the unextracted material.

40. The applicant suggests that the NI, consumed as a food supplement with a meal, can delay carbohydrate digestion in the small intestinal tract. The NI is purported to have the ability to inhibit the activity of the alpha-glucosidase enzyme so limiting the breakdown of carbohydrates and the subsequent formation of glucose and fructose (monosaccharides). The applicant states that undigested carbohydrates or disaccharides are then excreted rather than absorbed by the body therefore potentially offering assistance in weight control regimes.

41. For nutrition labelling purposes, 100g of the NI provides 60 g protein (minimum 55 g), no more than 1 g fat and 25 to 30 g of carbohydrate.

***Discussion**: Members accepted that the nutritional properties of the NI did not give cause for concern. The Committee noted that undigested fibre is likely to be fermented in the gut and referred to a recent review by the Scientific Advisory Committee on Nutrition (SACN)[1] which found limited evidence for the contribution of fibre to weight control.*

XII. Microbiological information on the novel food

Application dossier, pp 33-34

42. The production process for the NI involves sterilisation and filtration of the aqueous extract in order to minimise the risk of microbial contamination. The moisture content of the product in the powder form is <7% and the applicant is of the view that the potential for microbial growth is therefore limited. The manufacturing site has a certified HACCP system in place and all procedures comply with GMP as further assurance of the quality of the NI.

43. The applicant provides the results of an independent microbiological screen on the same three production batches of the NI discussed in Section I (Table XII.A in the dossier). No significant contamination with bacteria, mould, yeast or *E. coli* was detected in any of the batches tested.

***Discussion**: The Committee was of the view that the microbiological safety of the NI had been demonstrated and was reassured that the manufacturing site has a certified HACCP system in place and that all procedures comply with GMP.*

XIII. Toxicological information on the novel food

Application dossier, pp 35-52

44. The NI has undergone a number of toxicological analyses which were conducted by the applicant company. The NI was evaluated for acute oral toxicity in mice in an unpublished study where the LD_{50} was considered to be >5000 mg/kg body weight.

45. In a 28-day subacute/subchronic toxicity study of the NI, 4-week old rats were given doses of 250, 1000, and 2500 mg/kg body weight of the NI. No clinical signs or changes in body weight or food consumption related to the administration of the NI were observed. Although significant decreases in corpuscular haemoglobin and mean corpuscular volume for males in the 1000 mg/kg group were observed, these changes were thought to be unrelated to the test substance because no dose-dependant effects were noted. Although unilateral pelvic dilation was observed in the kidney of one male at the 2500 mg/kg dose group, these changes were considered to be spontaneous. No other significant changes were seen upon

[1] Narrative Synthesis of Health Effects of Potential Dietary Fibre Components - 13th October 2008 (http://www.sacn.gov.uk/pdfs/narrative_synthesis_paper_final.pdf)

histopathological examination. The NOAEL for the NI was considered to be more than 2500 mg/kg in males and females.

46. No studies that addressed the reproductive and developmental toxicity of the NI, or its mutagenic or genotoxic potential, were found in the published scientific literature. However, the applicant has provided a summary of a reverse mutation assay and an *in vivo* micronucleus test which indicated that the NI was neither mutagenic or genotoxic

47. The NI was also evaluated in an in vivo micronucleus test in rats which were administered the NI (10 mL/kg doses) by oral gavage on 2 successive days. Control animals received water at the same volume. A single 0.4 mg/mL dose of mitomycin C (MMC) was administered intraperitoneally (dosing volume 10 mL/kg) as the positive control substance. No dose-dependant increase was observed in the incidence of polychromatic erythrocytes with micronuclei among the NI-treated groups, whereas a significant increase in the incidence of polychromatic erythrocytes with micronuclei (4.92%) was observed in the positive control group treated with MMC.

48. The activity of the NI was evaluated in tissue, animal, and anti-infective in vitro assays as part of a pharmacological screen (PharmaScreen, MDS Panlabs Pharmocology Service, USA). The NI did not produce automatic signs or effects on the central nervous system, cardiovascular system, and gastrointestinal system. No metabolic effects were seen, nor were any indications of allergy or inflammation observed. No significant activity was observed at dose levels and concentrations tested. No microbiological pathogens were detected. A limited number of pharmacological studies in laboratory animals were also identified. No adverse effects were reported in rats and mice associated with the administration of the NI. The applicant provides a summary of studies examining the pharmacological effects of the NI in Table XIII.A. 6-1.

49. Human studies examining the effect of the NI on carbohydrate digestion following a meal in healthy, hyperlipidemic and diabetic subjects have also been summarised as part of this dossier. Patients were monitored for changes in various haemological and biochemical parameters, body weights and subjective side effects. The applicant is of the view that the absence of major adverse effects offers additional support for the safety of the NI. A summary of the clinical trials with the NI is provided in Table XIII.B.1-1. The applicant notes that no gastrointestinal effects were seen in clinical studies with the NI. The applicant also states that although soybeans can inhibit gastrointestinal proteases and therefore induce diarrhoea or abdominal pain, the NI did not demonstrate protease inhibition or cause this effect.

***Discussion**: The Committee was content that the toxicological assessment carried out by the applicant on the NI showed no evidence of adverse effects*

***Overall Discussion**: The Committee accepted that fermented black bean products are widely available on the market and have been consumed for many years in the form of soy sauce, miso and sake. Because the NI is obtained from fermented black bean using the same production process used for the production of black bean sauce, the Committee was content that no adverse effects are expected from consuming the NI. In addition the Committee was reassured that the fermentation conditions employed would be unlikely to give rise to the production of secondary metabolites.*

CONCLUSION

50. The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by CBC Co Ltd that the range of uses for its Touchi extract is acceptable, subject to the applicant's adherence to the proposed specification and the production parameters described above. The Committee also wishes to note that foods containing this novel ingredient should be labelled in accordance with existing legislation and should not make claims that are likely to mislead consumers.

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