

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

TOUCHI (FERMENTED BLACK BEAN) EXTRACT

**Issue**

An application has been submitted to the UK Competent Authority under the Novel Foods Regulation (EC) 258/97 for authorisation of Touchi extract derived from the fermentation of a variety of soya bean (*Glycine max*) by *Aspergillus oryzae*. The Committee is asked to advise whether the available data provides an adequate basis for a safety assessment, and if it recommends authorisation of this novel ingredient.

**Background**

Annex A p. i-iii

1. An application has been submitted by the Japanese company 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. In accordance with Article 6(3) of Regulation (EC) No 258/97, the UK has 3 months to prepare an initial assessment report on the above application. The European Commission will then circulate this initial assessment to the Competent Authorities in the other Member States for comment.
2. The application dossier is attached as **Annex A**.
3. Touchi extract is a protein-rich powder obtained by water extraction of small soybeans fermented with *Aspergillus oryzae*. The Novel Ingredient (NI) contains an alpha-glucosidase inhibitor and is intended to be consumed as a nutritional support by people who wish to slow the breakdown of carbohydrate following a meal. This 'carbohydrate blocking' property is thought to help people dieting to induce a feeling of satiety for longer after a meal.
4. This application 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. The applicant's NI 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). The requirements for a submission for this class are as follows:

I	<b>Specification of the NF</b>	X
II	<b>Effect of the production process applied to the NF</b>	X
III	<b>History of the organism used as the source of the NF</b>	X
IV	Effect of the genetic modification on the properties of the host organism	-
V	Genetic stability of the GMO	-
VI	Specificity of expression of novel genetic material	-
VII	Transfer of genetic material from GM microorganisms	-

VIII	Ability to survive in and colonise the human gut	-
IX	<b>Anticipated intake/extent of use of the NF</b>	X
X	<b>Information from previous human exposure to the NF or its source</b>	X
XI	<b>Nutritional information on the NF</b>	X
XII	<b>Microbiological information on the NF</b>	X
XIII	<b>Toxicological information on the NF</b>	X

5. The information presented in the dossier is structured accordingly and is considered below.
6. The Committee will wish to note that the application dossier will be published on the Agency's website for public consultation. Any comments received will be reported to the Committee in an addendum to this paper.

### **I. Specification of the novel food**

Annex A p. 3-13

7. The raw material used to prepare the 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 been used for hundreds of years in the production of soy sauce, miso and sake.
8. The NI is a light brown powder and the applicant has provided the following proposed specification which includes both nutrient and purity measures:

<b>Table I.B-1 Proposed Specification for Touchi Extract</b>		
<b>Parameter</b>	<b>Specification</b>	<b>Analytical Method</b>
<b>Characteristics</b>		
Appearance	Light brown powder	Visual
Taste	'Pleasant'	Taste
Fat	Max. 1%	Soxhlet extraction
Protein	Min. 55%	Kjeldahl method, AOAC 981
Water	Max. 7%	AOAC 925.1
$\alpha$ -Glucosidase inhibitor activity	IC <sub>50</sub> min. 0.025	Enzyme assay <sup>1</sup>
<b>Contaminants</b>		
Arsenic	Max. 10 $\mu$ g/kg	Atomic Absorption Spectroscopy
Aflatoxins	Max. 5 $\mu$ g/kg	HPLC
3-MCPD	Max. 50 $\mu$ g/kg	GCMS
Total heavy metals (expressed as lead)	Max. 20 $\mu$ g/kg	Na <sub>2</sub> S Colorimetric method
<b>Microbiological Requirements</b>		
Total bacteria count	$\leq$ 1000 cfu/g	USP23
Total mould and yeast count	$\leq$ 300 cfu/g	USP23
<i>Escherichia coli</i>	Negative /g	USP23

<sup>1</sup> Modified method of Miwa *et al.*, *Chem Pharm. Bull.*, 34:838, 1986

9. The applicant states that no pesticides are used. Results of a pesticide residue screen show none were present above the limits of detection.
10. The applicant has provided results of analysis for potentially toxic inherent constituents, external and process contaminants for three non-consecutive batches of the NI which they view to be representative of the commercial product to be marketed in the EU.
11. Heavy metal analysis for Lead and Cadmium show levels are within acceptable ranges (0.2  $\mu$ g/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.
12. At the proposed level of intake for the NI of up to 4.5g/day, 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.
13. None of the batches of the NI analysed for aflatoxins contained aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> above the limit of detection (<0.2  $\mu$ g/kg). The applicant states that as far as it is aware *Aspergillus oryzae* is not a source of Ochratoxin A and this is supported by the opinion of an expert of an accredited independent testing laboratory.
14. The level of 3-monochloropropane-1, 2-diol (3-MCPD) has been determined for three batches of the NI. The levels of 3-MCPD were found to be <30  $\mu$ g/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.

15. The applicant notes that one portion of a dish using 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 remain identical to the traditional fermented counterparts and 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.
16. Fermented black beans exhibit alpha-glucosidase inhibitory action and on extraction this activity is retained. The IC<sub>50</sub> 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.
17. 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 are provided by the applicant in confidential Section I.F.2.

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

Annex A p. 14-18

18. Fermentation using the fungus *Aspergillus oryzae* is a well established procedure used in the production of soy sauce, sake and miso. 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.
19. The applicant provides a basic overview of the fermentation process in Figure II.A.1-1, 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. The soybeans are then steamed and fermented in an aerobic environment using the fungus *Aspergillus oryzae*. The resulting fermented black bean is washed,

dried, screened for any foreign materials and packaged under vacuum until it is used in the extraction stage.

20. An overview of the extraction process of the NI is provided in Figure II.A.2-1. The fermented beans are milled and then suspended in boiling water to achieve extraction into aqueous phase. 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.
21. The applicant contends that there are no potential hazards associated with the processes employed in the production of the NI. In addition, they are of the view that no hazardous materials are formed under proper, regulated manufacturing conditions using *Aspergillus oryzae*.
22. 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 specification. If the final product does not meet the required specification, it is discarded.
23. The manufacture of the NI complies with principles laid out in Directive 93/43/EEC on the hygiene of foodstuffs at all stages of the production process. The applicant is of the view that 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.

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

Annex A p.19-22

24. 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 soybean plant is a species of legume and the soybeans obtained are considered oilseeds. 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.
25. The small soybean used in the production of the NI is 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

26. 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 adapted for use in fermentation processes.
27. Fermented black bean 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. Similarly, *Aspergillus oryzae* has a long history of safe use in food production spanning several hundred years and is also use in the production of livestock feed supplements.

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

Annex A p.23-27

28. The applicant intends that their NI will be used for nutritional support during a meal in order to hinder the digestion of carbohydrates in the small intestine tract in a similar way to food ingredients such as resistant starch or “starch blockers” currently on the market in the EU (See XI).
29. The NI will be available in food supplement 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 products will be clearly marked as providing a dose of the NI and will be labelled to indicate that the product should only be consumed with food. The applicant provides an example of an existing food supplement product available throughout the UK in sachet form for consumption as a stand alone drink or for addition to other beverages/foodstuff. The applicant anticipates that consumers will be familiar with this type of food supplement form and will follow the directions for use.
30. The applicant considers that, as the NI will only be available in clearly marked food supplement products, presentation to the consumer will be controlled. Capsules/tablets or sachets (e.g. tea or soup-type formulations) would be clearly marked as providing a dose of the NI where levels would not exceed 4.5 g per serving/dose per day.
31. 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.

32. The mean estimated total intakes 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<sup>th</sup> percentile of users) of the NI is 4.5 g and 36 g, respectively (equivalent to 1 or 8 servings approximately).
33. The applicant considers that it is likely to be an over estimate that consumers will be high level consumers of both “vegetable soups” and “tea, coffee and cocoa” and thus 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. The Secretariat notes that the estimates are based on the assumption that individuals who consume soups and/or tea and coffee always choose products that contain the novel ingredient. 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.
34. A NOAEL of 2,500 mg/kg bodyweight/day of NI (see para 49 below) equates to 150 g/day of the NI for a typical 60 kg adult. The average intakes of approximately 3 and less than half a serving for “tea, coffee and cocoa” and “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.

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

Annex A p.28-29

35. 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.
36. Soya beans are a common source of food allergy and are included in Annex IIIA of EU Directive 2003/89/EC regarding indication of ingredients in foodstuffs. 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.
37. 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. The Secretariat notes that this is irrelevant in terms of the requirement to label in accordance with Directive 2003/89/EC, unless the applicant applies for an exemption under that Directive.

38. The NI is currently approved as a Food for Specific Health Use (FOSHU) in Japan and the applicant has not received any consumer complaints of adverse effects relating to the consumption of its fermented black bean products, but has not indicated what mechanisms are in place to ensure that such complaints can be communicated.

## **XI. Nutritional information on the novel food**

Annex A p.30-33

39. 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.

40. 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 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. 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.

41. The isolated proteins typically have a Protein Digestibility Corrected Amino Acid Score (PDCAA) approaching 1, similar to milk proteins and egg white (FAO, 1991). Soy proteins are therefore considered "complete proteins" providing all the essential amino acids in sufficient quantities to meet nutritional needs. The whole soybean contains approximately 200 mg of isoflavones per 100 g and significant amounts are present in the majority of soy based products (USDA databases).

42. The applicant is of the view that the NI consumed as a food supplement with a meal can delay carbohydrate digestion in the small intestinal tract. Starch is digested by the action of amylase in saliva and the small intestinal juices to form maltose. Both the disaccharides maltose and sucrose are converted to their monosaccharide components by alpha-glucosidase at the mucosa in the small intestine. The NI has 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). Undigested carbohydrates or disaccharides are then excreted rather than absorbed by the body therefore potentially offering assistance in weight control regimes.

43. The clinical data indicate that the minimum dose at which the NI demonstrates alpha-glucosidase inhibitory activity is 0.3 g. (The Secretariat notes that the

inhibitory activity varies between batches – see paragraph 16 above – and the activity of the test material used in this study was not apparently recorded).

44. The applicant suggests that the effect of the NI on carbohydrate digestion is similar to that observed in a variety of other foods including indigestible dextrin, resistant starch, and alpha-amylase inhibitors. White kidney beans have been shown to hinder the digestion of complex carbohydrates and to play a role in weight control, although white kidney bean extract inhibits the amylase enzyme rather than alpha-glucosidase. These extracts are currently on sale in the EU and are marketed as “starch blockers”.
45. 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.

## **XII. Microbiological information on the novel food**

Annex A p.34-35

46. 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.
47. The applicant provides the results of an independent microbiological screen on the same three production batches of the NI discussed in Section I in Table XII.A. No significant contamination with bacteria, mould, yeast or *E-coli* was detected in any of the batches tested.

## **XIII. Toxicological information on the novel food**

Annex A p.36-46

48. The NI has undergone a number of toxicological analyses. The NI was evaluated for acute oral toxicity in mice in an unpublished study where the LD<sub>50</sub> was considered to be >5000 mg/kg body weight.
49. 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

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

50. 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
51. 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.
52. No chronic toxicity studies have been carried out with the NI.
53. 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 Pharmacology 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.
54. 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.

## **Committee Action Required**

55. The Committee is asked to consider whether the available data are adequate to determine whether the NI complies with the criteria for acceptance under the novel food regulation, namely:

- It does not present a danger to the consumer
- It does not mislead the consumer
- It is not nutritionally disadvantageous compared with foods which it might replace.

56. If so, the Committee is asked whether it is content to recommend approval for the NI to be used in the proposed food products.

57. If not, the Committee is invited to identify what further data should be provided.

**Secretariat  
July 2008**

### **Annexes attached:**

Annex A – Touchi extract application dossier (Restricted)  
(a non-confidential version of this dossier is publicly available via the ACNFP's website  
[www.acnfp.gov.uk](http://www.acnfp.gov.uk))

**ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES**

**RESTRICTED**

Application for the approval of Touchi extract

**Secretariat  
July 2008**