European Food Safety Authority

EFSA Journal 2011;9(5):2136

SCIENTIFIC OPINION

Scientific Opinion on the safety of a 'fermented black bean extract (Touchi extract)' as a Novel Food ingredient [1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to carry out the additional assessment for 'fermented black bean extract (Touchi extract)' as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States. Touchi extract is obtained by hot water extraction of small soybeans (*Glycine max*) fermented with *Aspergillus oryzae.* Hot water extraction of 15 g of fermented black beans yields 4.5 g of Touchi extract. The Panel considers that data on the composition, production process and stability do not raise concerns. The applicant intends to market Touchi extract in food supplements to adults and proposes one dose of 0.3 g Touchi extract to be consumed with every meal of the day. The applicant anticipates an average intake of 0.9 - 1.2 g Touchi extract per day and proposes a maximum intake level of 4.5 g per day. The applicant provided data that indicate that the typical serving size of traditional fermented black bean products contains approximately 15 g of fermented black beans. The documentation on the safety of Touchi extract is based mainly on the history of the source of the novel food, on the significant human consumption of traditional fermented black bean products in and outside of Europe, data on the production process and on the compositional similarity between fermented black beans and Touchi extract. Toxicological and clinical data provide limited evidence for the safety of Touchi extract and do not raise concerns. The Panel concludes that the 'fermented black bean extract (Touchi extract)' is safe at the proposed conditions of use.

KEY WORDS

'Fermented black bean extract', Touchi extract, novel food, ingredient

---

[1] On request from the European Commission, Question No EFSA-Q-2009-00765, adopted on 08 April 2011.

[2] Panel members: Carlo Virgilio Agostini, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Yolanda Sanz, Seppo Salminen, John (Sean) J Strain, Stephan Strobel, Inge Tetens, Daniel Tome, Henk van den Berg, Hendrik van Loveren and Hans Verhagen.
Correspondence: nda@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank for the preparatory work on this scientific opinion: The members of the Working Group on Novel Foods: Karl-Heinz Engel, Ines Golly, Marina Heinonen, Pagona Lagiou, Rosangela Marchelli, Bevan Moseley, Monika Neuhäuser-Berthold, Annette Pöting, Seppo Salminen, Hendrik Van Loveren, Hans Verhagen and Ivonne Rietjens.

Suggested citation: EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the safety of ''fermented black bean extract' (Touchi)' as a Novel Food ingredient. EFSA Journal 2011;9(5):2136. [20 pp.]. doi:10.2136/j.efsa.2011.2136. Available online: www.efsa.europa.eu/efsajournal.htm

SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to carry out the additional assessment for ''fermented black bean extract’ (Touchi extract)' as a food ingredient in the context of Regulation (EC) No 258/97 taking into account the comments and objections of a scientific nature raised by Member States.

Touchi extract is obtained by hot water extraction of small soybeans (*Glycine max*) fermented with *Aspergillus oryzae*. The proposed novel food ingredient is a powder containing a minimum of 55 % protein, no more than 1 % fat, 25 to 30 % of carbohydrate and approximately 7.8 % of ash. The applicant provided sufficient information on inherent constituents of potential concern and external contaminants. The fermentation process is a well established procedure employed in the production of traditional fermented black bean products such as soy sauce, sake and miso. Both the small soybeans and fermented black bean products such as soy sauce, sake and miso have a significant history of human consumption. The fermented black beans are milled, suspended in water, heated and extracted into the aqueous phase. Following centrifugation and filtration the dialysate is concentrated and spray dried to give the final product Touchi extract. Results from stability studies indicate that Touchi extract is stable over 36 months at room temperature.

The applicant indicated that no major chemical modification of the fermented black beans is involved during the extraction with water, despite the elevated temperature. Although only the water soluble components will probably be extracted, the Panel considers that the composition and the nature of the nutrients remain similar to the traditional fermented black bean products. The chromatograms obtained from HPLC analysis of ‘fermented black bean extract’ and fermented black bean paste are very similar and exhibit peaks consistent with low molecular weight peptides and amino acids. The applicant indicates that the water extraction of 15 g of fermented black beans yields 4.5 g of Touchi extract, which is the maximum dose per day proposed by the applicant.

The Panel considers that the data on the composition and on the production process do not raise concerns.

The applicant intends to market Touchi extract in food supplements such as tablet/capsule or sachets (e.g. tea or soup-style formulations), for the delivery as a stand-alone drink or for addition to other beverages/foodstuffs. The applicant proposes that food supplements should be consumed as a dose of 0.3 g consumed with every meal of the day. Considering usual meal intakes of 3 - 4 per day the applicant anticipates an average intake of 0.9 - 1.2 g Touchi extract per day and proposes a maximum intake level of 4.5 g per day. The applicant indicates that adults who wish to inhibit the digestion of carbohydrates for weight management purposes are the target population. The applicant provided data that indicate that the typical serving size of fermented black bean products contains approximately 15 g of fermented black beans. An intake estimate based on UK consumption data suggests that a daily intake of 15 g fermented black beans corresponds to the 90th percentile intake estimate for adult people in the UK. The Panel notes that the intake of corresponding proposed maximum dose of 4.5 g Touchi extract would be within the high percentile of the provided intake estimate.

The toxicological data base on the ‘fermented black bean extract’ is limited to data on acute toxicity, genotoxicity and a 28-day sub-chronic study in the rat, which established a NOAEL of 2500 mg/kg bw/day, the highest dose tested, equivalent to 150 g/day for a 60 kg adult. The results from the limited toxicological evaluation do not raise concerns.

The Panel notes that the clinical studies with, in total, approximately 185 subjects were primarily designed to demonstrate efficacy of the Touchi extract. However, some safety parameters such as haematological and biochemical parameters and subjective side effects (complaints) were studied as well. No adverse effects in subjects were reported from these studies. The Panel considers that these studies provide very limited evidence for human safety at the proposed maximum dose levels.

Documentation on the safety of Touchi extract is based mainly on the history of the source of the novel food, on the significant human consumption of traditional fermented black bean products in and outside of Europe, data on the production process and on the compositional similarity between fermented black beans and Touchi extract. Toxicological and clinical data provide limited evidence for the safety of the Touchi extract and do not raise concerns.

The Panel concludes that the ‘fermented black bean extract (Touchi extract)’ is safe at the proposed conditions of use.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 8 July 2008, the company CBC Co. Ltd. submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to place on the market a ''fermented black bean extract' ' as a novel food ingredient.

On 28 January 2009, the competent authorities of the United Kingdom forwarded to the Commission their initial assessment report, which came to the conclusion that the ‘fermented black bean extract’ may be placed on the market.

On 5 February 2009, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) No 258/97.

The concerns of a scientific nature raised by the Member States can be summarized as follows:

- The applicant must provide a more precise botanic definition of the variety of soya used, since this name is normally used to refer to several different types of plant.
- The strains of *A. oryzae* used for industrial applications generally originate from collections. The applicant fails to specify the origin of the strain used and recommends making sure that the strain does not revert to *A. flavus* over the course of time, resulting in the production of aflatoxins.
- Specification data of the Touchi extract's main ingredients cover only some 60 % of the product. Additional data in nutritional information section is based on testing of only one batch.
- The maximum heavy metal, dioxin and PCB levels of the product must be specified and further details provided concerning the presence of these contaminants. Inappropriate information on total heavy metals (not in accordance with European Pharmacopoeia, insufficient information on the applied method). Heavy metal concentrations in the product in question are higher than listed in the specification.
- The possible presence of mycotoxins other than aflatoxins was not investigated, even though the fungus *Aspergillus* is known to produce different kinds of mycotoxins.
- The applicant intends to market Touchi extract to consumers who want lose weight. Due to insufficient safety data, it should not be recommend for children, pregnant and lactating women.
- The toxicological information is not sufficient for a comprehensive evaluation of Touchi extract. Further information on the specification of the product's composition and the possible presence of toxicologically relevant ingredients should be requested, with the concentrations of kojic, cyclopiazonic and ß-nitropropionic acid and biogenic amines, especially histamines and ethyl carbamate in relation to other soy products, being of particular interest.
- Levels of antinutritive substances including tannic acid, trypsin inhibitor and isoflavonoids, present in soya bean before and after fermentation should be specified and addressed.
- The active inhibitor of the α-glucosidase must be identified and described so that it can be specifically apportioned to different foodstuffs if necessary.

- If larger amounts of disaccharides (inhibition of intestinal α-glucosidase) are not digested and absorbed in the small intestine, intestinal discomfort (bloating, diarrhoea) can arise. Possible effects on the composition of the microbiota have not been addressed.
- The relevance of the clinical studies is questionable, due to their small size, number of end-points, low dose levels, administration scheme.
- Either more details on the micro-nutrient composition of the NF or a sub-chronic toxicity study required.
- Potential allergic reactions in relation to *Aspergillus spp.* should be addressed.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for the 'fermented black bean extract' as food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of scientific nature in the comments raised by the other Member States.

ASSESSMENT

In accordance with the Commission Recommendation 97/618/EC 'fermented black bean extract', in the provided dossier called 'Touchi extract', is allocated to Class 2.1 'complex (non-GM derived) novel food, the source having a history of food use in the Community'. The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the competent authority of the United Kingdom (UK), the concerns and objections of the other Member States and the responses of the applicant to these questions and those of the UK authority. The data are required to comply with the information required for novel foods of class 2.1 i.e. structured schemes I, II, III, IX, X, XI, XII and XIII. It is noted that the novel food is intended by the applicant to be marketed as food supplements for body weight management purposes. This assessment concerns only safety in relation to consumption of the novel food ingredient Touchi extract, and is not an assessment of the efficacy with regard to any claimed benefit.

1. Specification of the Novel Food (NF)

'Fermented black bean extract' (Touchi extract) is a protein-rich powder obtained by water extraction of small soybeans (*Glycine max*) fermented with *Aspergillus oryzae.* The extract contains an α-glucosidase inhibitor. The specifications for 'fermented black bean extract' proposed by the applicant are presented in Table 1.

Table 1 : Specification for 'fermented black bean extract' as proposed by the applicant.

Parameter	Specification	Analytical method
Characteristics		
Appearance	Light brown powder	Visual
Fat	Max. 1%	Soxhlet extraction
Protein	Min. 55%	Kjeldahl method, AOAC 981
Water	Max. 7%	AOAC 925.1
Ash	Max. 10 %	Direct ashing method
Carbohydrate	Min. 20 %	Subtraction method
α-glucosidase inhibitory activity	IC50 min 0.025 mg/mL	Enzyme assay[(a)]
Soy isoflavone	0.3 g/100 g	Ministry of Health, Labour and Welfare Japan (2006)
Contaminants		
Arsenic	Max. 10 mg/kg	ICP-MS
Cadmium	Max 0.2 mg/kg	ICP-MS
Lead	Max. 0.2 mg/kg	ICP-MS
Aflatoxins	Max. 5 μg/kg	HPLC
3-MCPD	Max. 50 μg/kg	GCMS
Microbiological Requirements		
Total bacteria count	≤ 1000 cfu/g	USP23

Total mould and yeast count	≤ 300 cfu/g	USP23
Escherichia coli	Negative/g	USP23

(a): Modified method of Miwa et al., Chem. Pharm. Bull., 34: 838, 1986.

The applicant indicated, however, that 'fermented black bean extract' can be considered as providing approximately 60 g protein (minimum 55 g), no more than 1 g fat and 25 to 30 g of carbohydrate per 100 g. Furthermore analysis of the composition of a single batch of 'fermented black bean extract' indicated the presence of 7.8 g ash/100g. If 7.8 g ash/100 g is added to the specifications, about 93-98 % of the material is accounted for.

The Panel notes that the specifications as provided by the applicant do not include levels of a variety of ingredients known to be present in soybean which might be relevant from a safety point of view, including for example kojic, cyclopiazonic and beta-nitropropionic acid and biogenic amines, especially histamines and ethyl carbamate, as well as levels of antinutritive substances including tannic acid, trypsin inhibitor and isoflavones. Isoflavones are flavonoids with structural similarities to oestrogens. The applicant indicated that the whole soybean contains approximately 200 mg of isoflavones per 100 g, but actual levels present in the Touchi extract are not provided. Taking into account that the preparation process does not concentrate isoflavones and the intended maximum intake of 4.5 g Touchi extract per day, the content of isoflavones is not of concern.

The Panel asked the applicant for detailed compositional information, such as the quality of the protein/peptide content, qualitative and quantitative data on the carbohydrate fraction (other than calculating the content of carbohydrates by 100 % minus other constituents), on secondary plant constituents such as kojic, cyclopiazonic and beta-nitropropionic acid and biogenic amines, especially histamines and ethyl carbamate, as well as levels of other potential anti-nutritive substances including tannic acid and trypsin inhibitor. The Panel also asked for data on micronutrients, if available, because these would be supportive for the overall characterisation of 'fermented black bean extract'.

The HPLC chromatograms of a sample of the 'fermented black bean extract' and of a fermented black bean paste were shown to be similar, exhibiting peaks consistent with amino acids and low molecular weight peptides. However neither amino acids nor peptides were identified. It is noted that amino acids and peptides are referred to in Tables 1 and 2 as "proteins". Data obtained by SDS Gel-Electrophoresis indicate that upon fermentation with *A. oryzae* the levels of peptides with a molecular weight smaller than 20 kDa are significantly increased as compared to the starting raw soybeans, with a concomitant decrease of the larger peptides. Specifically, on fermentation of soybean meal no peptides greater than 60 kDa in size were detected, compared to ca. 22 % in the starting material (Hong et al., 2004).

The applicant provided data on compositional analysis of three batches of 'fermented black bean extract' which are presented in Table 2.

Table 2: Nutrient profiles for three batches of 'fermented black bean extract' as provided by the applicant.

Nutrient	Specification	Batch results (% w/w total composition)		
		Batch 1	Batch 2	Batch 3
Fat	Max. 1 %	0.2	0.1	0.2
Protein	Min. 55 %	63.1	66.3	65.0
Carbohydrate	Min. 20 %	25.3	21.5	23.6
Water	Max. 7 %	3.5	3.2	3.9
Ash	Max. 10 %	7.9	8.9	7.3

The applicant also provided analytical data on potentially toxic inherent constituents and undesirable substances for three batches of 'fermented black bean extract'. These data are presented in Table 3.

Table 3: Analytical data on constituents of possible concern for three batches of 'fermented black bean extract' as provided by the applicant.

Parameter	Unit	LOD	Batch 1	Batch 2	Batch 3
Kojic acid	mg/kg	5	ND	ND	ND
Cyclopiazonic acid	mg/kg	5	ND	ND	ND
Beta-nitropropionic acid	mg/kg	25	ND	ND	ND
Histamines	mg/100 g	0.5	ND	ND	ND
Ethyl carbamate	mg/kg	0.1	ND	ND	ND
Tannic acid	g/100 g	-	2.78	2.72	2.75

ND = not detected, LOD = Limit of Detection.

It is established that soybeans inhibit gastrointestinal proteases, such as trypsin that can lead to diarrhoea and/or abdominal pain (Fujita et al. 2001b). The applicant provided a study which indicated that fermentation of soybeans with *A. oryzae* effectively reduced the trypsin inhibitory property of soybeans (Hong et al., 2004). The applicant indicates that this is supported by the fact that a sample of 'fermented black bean extract' was screened for inhibition of a range of enzymes and no trypsin inhibitory activity was observed.

The Panel considers that the applicant provided sufficient information on inherent constituents of potential concern.

The IC50 for inhibition of α-glucosidase for three batches of 'fermented black bean extract' ranged from 0.05 to 0.55 mg/mL. The nature of the α-glucosidase inhibitor has not been defined. According to the applicant an inhibition of intestinal α-glucosidase is only found when the black beans have been fermented with *Aspergillus spp*. but not in raw or boiled beans.

Aflatoxins are produced by certain species of *Aspergillus*, the fungus employed in the fermentation of the small soybeans to form 'fermented black bean extract' . Three batches of 'fermented black bean extract' were analysed for aflatoxins B1, B2, G1, and G2. None of the batches were found to contain these mycotoxins above the limit of detection (< 0.2 µg/kg). The applicant indicated that as far as he is aware *A. oryzae* is not a source of Ochratoxin A (Fennel, 1976; Stoloff *et al.*, 1977).

Information on the batch testing was provided for heavy metals, dioxins and dioxin-like PCBs, polycyclic aromatic hydrocarbons (PAHs), 3-monochloropropane-1,2-diol (3-MCPD) and pesticides. The applicant stated that no pesticides are used at any stage of the production process or are used on other crops in regions in which the small soybean is grown.

The Panel considers that the information provided on the composition, specification and data from batch testing do not raise concerns. With regards to contaminants, the Panel notes that the novel food has to comply with existing legislation.

Stability of the Bulk Powder

The applicant provided results from stability studies that indicate that bulk powder of 'fermented black bean extract' is stable over 36 months at room temperature as established by measuring the taste, appearance, and α-glucosidase inhibitory activity at regular intervals.

The stability of 'fermented black bean extract' has also been monitored over time in typical formulations both at room temperature and at elevated temperature (accelerated studies). Based on taste, appearance, and α-glucosidase inhibitory activity, formulations of 'fermented black bean extract' appear stable at room temperature over 24 months and at 55° C over 6 months, respectively.

The applicant also indicated that generally, foods formed by fermentation have a higher risk of containing microorganisms and that therefore a pasteurisation step is included in the final stages of product manufacture. In addition, preparation of the product in the powder form lowers the amount of moisture to < 15 % thereby reducing the potential for microbial growth and increasing the shelf-life of the product. Limits on microbial growth are included in the product specification.

2. Effect of the production process applied to the NF

Fermented black beans are derived from the small soybean grown in the Sichuan province of China. The soybeans are steamed and fermented using the fungus *A. oryzae*. The applicant indicated that fermentation using *A. oryzae* is a well established procedure employed in the production of soy sauce, sake and miso (Wood, 1977) and that it has been established that no hazardous materials are formed under proper, regulated manufacturing conditions using *A. oryzae* (U.S. EPA, 1997). The applicant also indicated that *A. oryzae* use would be regulated in the future under pending regulation in the EU establishing a common authorisation procedure for food additives, food enzymes and food flavours (European Parliament and the Council of the European Union, 2006a, b).

The fermented black beans are milled, suspended in water, heated at boiling temperature and extracted into the aqueous phase. Following centrifugation and filtration the dialysate is concentrated and spray dried to give the final product, 'fermented black bean extract', as a pale brown powder.

No major chemical modification of the fermented black beans is involved during the extraction with water, despite the elevated temperature. Although only the water soluble components will probably be extracted, the Panel considers that the composition and the nature of the nutrients remain similar to the traditional fermented black bean products.

The applicant noted that the conditions used for production of the 'fermented black bean extract' are consistent with the conditions used in the production of black bean sauce. The UK Advisory Committee on Novel Foods and Processes (ACNFP) requested further information on the similarity of the heat- treated 'fermented black bean extract' to black bean sauce. Upon this request the applicant responded that although the production of the 'fermented black bean extract' involved two separate heat treatments, the second treatment is the sterilization step and is a controlled treatment performed over a short period of time (135°C, 15 sec). 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 opinion that any general effects of heat treatment on the protein components will be consistent between the sauce and the extract.

In response to a further request from the ACNFP, the applicant provided the results of a HPLC analysis to demonstrate the effect of fermentation and hot water treatment on the "protein" (peptides and amino acids) content of the product although neither peptides nor amino acids were identified.

The Panel noted the subsequent aqueous extraction process for the production of the 'fermented black bean extract' and required additional compositional data that could demonstrate the similarity. The applicant indicated that the chromatograms for 'fermented black bean extract' and fermented black

bean paste were overlaid and shown to be very similar, with no significant differences between the chromatograms of the two products, although the compounds were not identified.

The Panel acknowledged that the applicant's arguments on the similarity between fermented black bean sauce and 'fermented black bean extract' are based on the commonly shared fermentation process and data obtained by SDS- Gel Electrophoresis.

The applicant indicated that one portion of a dish using traditional black bean sauce would generally contain 15 g of fermented black beans, which on extraction with water, corresponds to 4.5 g of 'fermented black bean extract' . Upon request the applicant confirmed that water extraction of 15 g of fermented black beans yields 4.5 g of 'fermented black bean extract'.

On this basis, the Panel considers that the proposed maximum daily intake of 4.5 g per day of 'fermented black bean extract' is equivalent to one serving of a typical dish containing the traditional form of fermented black beans.

The Panel concludes that the production process is sufficiently described by the applicant and does not raise concerns.

3. History of the organism used as the source of the NF

The raw material used to prepare 'fermented black bean extract' is the small soybean (*Glycine max*.), which has been extensively used in the Sichuan province of China for centuries where it is known as the small yellow bean. The small soybean plant is a species of legume.

The fermentation of the small soybeans is performed using the fungus *A. oryzae*. The *Aspergillus* genus is separated into groups based on morphological characteristics of the individual species and *A. oryzae* is a member of the *A. flavus* group. *A. oryzae* has undergone extensive selection over the years in order to produce the current strains adapted for safe and suitable use in fermentation processes.

4. Anticipated intake/extent of the use of the NF

The applicant intends to market 'fermented black bean extract' in food supplements such as tablet/capsule or sachets (e.g. tea or soup-style formulations), for the delivery as a stand-alone drink or for addition to other beverages/foodstuffs. The applicant proposes the food supplements should be taken in a dose of 0.3 g consumed with every meal of the day. Considering usual meal intakes of 3 - 4 per day the applicant anticipates an average intake of 0.9 - 1.2 g per day and proposes a maximum intake level of 4.5 g per day. All products would be labelled with an indication of the dose. The applicant indicates that adults, who wish to inhibit the digestion of carbohydrates for weight management purposes, are the target population. The product would not be marketed to children or pregnant and lactating women, although the applicant reasoned that occasional consumption of Touchi extract under the conditions proposed (typically around 1 g per day) would not pose a safety concern to children or to pregnant and lactating women.

Since 4.5 g of 'fermented black bean extract' could be considered equivalent to 15 g of fermented black beans in a traditional product on the market, the applicant provided an intake estimate of traditional fermented black beans products for the United Kingdom (UK) population in order to show that the maximum proposed dose of 4.5 g would be comparable to the high percentile intake estimate of traditional fermented black bean products by UK consumers. For this intake estimate data from the National Diet and Nutrition Survey (NDNS) were employed:

Data of adults aged 16 to 64 years were collected in the National Diet and Nutrition Survey (NDNS) of 2000 - 2001 (Office for National Statistics, 2005), of children aged 1½ to 4½ years in the NDNS of 1992 - 1993 (UKDA, 1995), and of young people and teenagers aged 4 to 18 years in the NDNS of

1997 (UKDA, 2001). Weighted 4- or 7-day food records for individuals were selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors as the primary sampling unit. Individuals were considered users if they consumed one or more food fermented black beans products on at least one of 7 survey days.

From the data sets all food codes representing fermented black beans or mixed foods and sauces prepared from fermented black beans were selected from the database. For the mixed foods, the composition of the food was adjusted in order to accurately represent the percentage of the recipe accounted for by fermented black beans.

Only a small percentage of consumers (< 2 %) was identified in the NDNS survey respondents and therefore the intake on a total population basis is considered not relevant. The estimated mean intake limited to consumers of traditional products with fermented black bean products ranged between 3.6 and 18.6 g/person/day. When the high level (97.5th percentile) consumers were considered, the lowest intake was observed to occur in young people at 4.9 g/person/day and the highest intake was observed to occur in male teenagers at 37.2 g/person/day.

The applicant noted that mean and high percentile intake estimates based on sample sizes of less than 30 and 160, respectively, may not be considered statistically reliable due to the limited sampling size (LSRO, 1995). As such, the reliability of the above estimates for the intake of fermented black beans based on the consumption of these foods is questionable.

In addition, the applicant provided recipes of Asian dishes which suggest that the typical serving size of fermented black bean products (such as paste or sauce) would approximately correspond to 15 g of fermented black beans, which is the equivalent to the maximum proposed intake of 4.5 g Touchi extract.

5. Information from previous exposure to the NF or its source

The applicant indicated that black beans fermented by *A. oryzae* have been widely used in China for the past 1000 years as a traditional seasoning and people are familiar with black beans as a protein source that can be preserved by drying without impairment of the nutrient content. Fermented black beans are also commonly referred to as „Touchi" in Japan (Fujita and Yamagami, 2007) and Douchi in Taiwan and China (Chen *et al*., 2006).

Foods obtained from fermented soybeans in general have had significant role in the diet of the Eastern Asian population for centuries (Hesseltine and Wang, 1980, Hui, 1991; Murooka and Yamshita, 2008). The wide availability of these products in Asian supermarkets provides qualitative evidence for a significant consumption by the population. Traditional Asian foods are Chinese soy sauce, Japanese shoyu, Japanese miso, Indonesian kecap, Taiwanese inyu, Korean kan-jang, Philippino tasoso and Japanese sake (Steinkraus, 2002).

According to the applicant's information, fermented black beans have also been consumed in Europe for many years in Chinese dishes containing "black bean sauce" or involving "black bean paste" (prepared by crushing fermented black beans) as a seasoning, typically at levels of around 15 g per serving. Limited evidence has been identified confirming the production of foods using *A. oryzae* or the production of salt savoury flavoured amino acid/peptide sauces and pastes in the United States and Europe (US EPA, 1997; Steinkraus, 2002).

Information provided by the applicant indicates that 'fermented black bean extract' has been approved as a Food for Specific Health Use (FOSHU) in Japan.

6. Nutritional information on the NF

The NF is purported to have the ability to inhibit the activity of the α-glucosidase enzyme so limiting the breakdown of carbohydrates and the subsequent formation of glucose and fructose (monosaccharides). On request of EFSA, the applicant provided data on the activity of α-glucosidase of samples from five different commercial fermented black bean products and of three batches of the Touchi extract (CBC, 2011). The IC50 (g/L) values of α-glucosidase of the of Touchi extract samples were within the values of the traditional products with fermented black beans.

'Fermented black bean extract' provides approximately 60 g protein, not more than 1 g fat and about 25 to 30 g of carbohydrate per 100 g.

Based on the information provided on the composition and the proposed use level, the Panel considers an intake of 4.5 g 'fermented black bean extract' per day is not nutritionally disadvantageous.

7. Microbiological information on the NF

The production process of 'fermented black bean extract' involves filtration and sterilisation of the aqueous extract in order to minimise the risk of microbial contamination. In addition, the moisture content of the product in the powder form is < 7 % so limiting the potential for microbial growth. The results of an independent microbiological screen on three production batches of 'fermented black bean extract' revealed that no pathogen contamination was detected in any of the batches tested (total bacterial count less than 1000 cfu/g; total mould and yeast count less than 300 cfu/g and *Escherichia coli* negative/g).

8. Toxicological information on the NF

8.1. Acute Toxicity

The applicant reported that 'fermented black bean extract' was evaluated for acute oral toxicity in mice in an unpublished study in accordance with OECD Guidelines for the Testing of Chemicals 401 (Japan Food Research Laboratories 2000a). Oral administration of 5000 mg/kg bw/day of 'fermented black bean extract' to male and female ICR mice caused no deaths. No clinical abnormalities or significant body weight changes were observed. No remarkable changes were found in any organ at necropsy. The LD50 was considered to be > 5000 mg/kg bw.

8.2. Sub-acute Toxicity

A 28-day toxicity study with 'fermented black bean extract' was performed according to national (Japanese) guidelines by Fujita and Yamagami (2007) in male and female SPF rats of Crl:CD(SD) strain, obtained from Charles River Japan, Inc. at the age of 4 weeks. Ten healthy animals of each sex were randomly assigned to each of four groups. The rats were given doses of 0 (control) 250, 1000, and 2500 mg/kg bw 'fermented black bean extract' per day.

No clinical signs or changes in body weight or food consumption related to the administration of 'fermented black bean extract' were observed. No abnormal changes were observed in any urinalysis parameters in treated animals as compared with the control animals.

A statistically significant decrease was seen in mean corpuscular haemoglobin and mean corpuscular volume for males in the 1000 mg/kg bw group, while mean corpuscular haemoglobin concentration was statistically increased in these animals. However, these changes were considered to be unrelated to the test substance, because no dose-dependent effects were noted. No other significant changes were seen in haematological parameters.

Similarly, a statistically lower chloride value for males in the 1000 and 2500 mg/kg groups, and a lower γ-glutamyltransferase (γ-GTP) value for females in the 250 mg/kg group were observed. These changes were likewise judged by the authors to be unrelated to the test substance, because they were unrelated to dose or considered a mild change within the range of background data. No other significant effects on clinical chemistry parameters were noted.

Although a statistically lower value was seen in the relative thymus weight for males in the 1000 mg/kg bw group (123 ± 24 *vs*. 156 ± 33 mg %; $p < 0.05$ by Dunnett's test), this change was not considered to be related to the administration of 'fermented black bean extract' since it was unrelated to dose and was within the range of background data. Unilateral pelvic dilation was observed in the right kidney of one male at the 2500 mg/kg bw dose group at necropsy. Upon histopathological examination, slight interstitial mononuclear cell infiltration and unilateral dilatation of pelvis in the kidney were observed. These changes were observed in only one animal and thus considered by the study authors to be of spontaneous origin. No other changes were seen upon pathological or histopathological examination.

The applicant concluded that because no toxic changes were observed at 2500 mg/kg bw/day in both sexes, the NOAEL for 'fermented black bean extract' was considered to be more than 2500 mg/kg bw in males and females.

The Panel notes that no 90-day study on 'fermented black bean extract' was provided.

8.3. Reproductive/Developmental Toxicity

No studies that addressed the reproductive and developmental toxicity of 'fermented black bean extract' were available.

8.4. Chronic Toxicity

No studies that addressed the chronic toxicity of 'fermented black bean extract' were provided.

8.5. Genotoxicity

The applicant indicated that *in vitro* mutagenic potential of 'fermented black bean extract' at 313, 625, 1250, 2500 and 5000 µg/plate with and without metabolic activation by a S9 mix was evaluated in a reverse mutation assay with *Salmonella enterica* var. Typhimurium strains TA100, TA98, TA1535, and TA1537 and *Escherichia coli* WP2 *uvrA.* No evidence of mutagenic effects was observed with 'fermented black bean extract' in any strain with or without metabolic activation (Fujita and Yamagami, 2007; Japan Food Research Laboratories, 2000b).

'Fermented black bean extract' was also evaluated in an *in vivo* micronucleus test according to national (Japanese) guidelines in Male SPF rats of Crl:CD(SD) rats at dose levels of 500, 1000 and 2000 mg/kg bw/day (Fujita and Yamagami, 2007). Rats were administered 'fermented black bean extract' by gavage on 2 successive days. Control animals received water at the same volume. The results of the study reveals that the mean incidence of polychromatic erythrocytes with micronuclei in the 'fermented black bean extract' - treated groups was equivalent to or less than in the negative control group (0.14, 0.09, and 0.06 % in the 500, 1000, and 2000 mg/kg bw groups, respectively, and 0.13 % in the negative control group). The mean frequency of polychromatic erythrocytes to the total erythrocytes was 48.3, 49.2, and 47.4 % in the 500, 1000, and 2000 mg/kg bw groups, respectively, and 47.8 % in the negative control group.

The Panel considers that the results do not provide cause for concern.

8.6. Other Studies

The applicant indicated that the activity of 'fermented black bean extract' was evaluated in tissue, animals, and anti-infective *in vitro* assays as part of a pharmacological screen (PharmaScreen®, MDS Panlabs Pharmacology Services, USA). 'Fermented black bean extract' did not produce autonomic signs or effects on the central nervous system, cardiovascular system, and gastrointestinal system. No metabolic effects were seen, nor were indications of allergy or inflammation observed. No significant activity was observed at dose levels and concentrations tested. No microbiological pathogens were detected (MDS Panlabs, 2000).

The applicant also stated that a limited number of pharmacological studies in laboratory animals were identified, and that no adverse effects associated with the administration of 'fermented black bean extract' were reported in rats and mice in these studies (Fujita and Yamagami, 2001; Fujita et al., 2001a; Fujita et al., 2005)

The Panel notes that these studies were not designed to demonstrate the safety of 'fermented black bean extract'.

8.7. Human Data

Clinical studies were provided which were designed primarily to examine the effects of Touchi extract on carbohydrate digestion (Nippon Supplement Inc Research and Development, 1999a; Nippon Supplement Inc Research and Development, 1999b; Hiroyuki et al., 2001; Fujita et al., 2001a; Fujita et al., 2001b; Fujita et al. 2003; Fujita et al. 2005). The clinical studies also evaluated common haematological and biochemical parameters and subjective side effects (complaints).

Only synopses were provided of a study that evaluated a single bolus of 10 g Touchi extract in 3 healthy male subjects (Nippon Supplement Inc Research and Development, 1999a) and of a study which tested 3 x 1 g consumed by healthy male subjects over 12 weeks (Nippon Supplement Inc Research and Development, 1999b). Another study with, in total, eight diabetic subjects evaluated the glycaemic response of a single dose of 0.1, 0.3, 1.0, 3.0, and 10.0 g (Fujita et al., 2001a). The remaining clinical studies tested doses at 1 (3 x 0.3) or 3 (3 x 1) g Touchi extract per day consumed with meals over 3 to 6 months, in diabetic or hypertriglyceridaemic subjects.

The Panel notes that these clinical studies were primarily designed to demonstrate efficacy of Touchi extract. The Panel notes the limitations of these studies with regards to demonstrating human safety of Touchi extract at the proposed maximum intake levels (4.5 g/day), the study population, endpoints and documentation. However some safety parameters such as haematological and biochemical parameters and subjective side effects (complaints) were studied and no adverse effects in subjects were reported from these studies.

The Panel considers that these studies provide limited evidence for the human safety of Touchi extract at the proposed uses.

8.8. Allergenicity

Soybeans are a common source of food allergenicity and are included in Annex IIIa of EU Directive 2003/89/EC regarding the indication of ingredients present in foodstuffs (European Parliament and the Council, 2003), which states that all products containing soybeans and products thereof as an ingredient must be clearly labeled.

In addition potential allergenic risk from *A. oryzae* cannot be ruled out, but this risk is expected not to be higher than of consumption of other *Aspergillus* derived products.

The Panel noted that no studies on allergenicity of 'fermented black bean extract' were provided.

DISCUSSION

No major chemical modification of the components is involved during extraction of 'fermented black bean extract' with water, despite the extraction is performed at elevated temperature. Although only the water soluble components will be extracted, the Panel considers that the composition and the nature of the nutrients remain similar to the traditional fermented black bean products. The chromatograms obtained from HPLC analysis of 'fermented black bean extract' and fermented black bean paste were very similar and exhibit peaks consistent with low molecular weight peptides and amino acids. The Panel acknowledged that the applicant's arguments on the similarity between fermented black bean sauce and 'fermented black bean extract' are based on the commonly shared fermentation process and data obtained by SDS-Gel Electrophoresis. The applicant indicates that the water extraction of 15 g of fermented black beans yields 4.5 g of Touchi extract, which is the maximum dose per day proposed by the applicant. The Panel considers that the data on the composition and on the production process do not raise concerns.

The applicant intends to market Touchi extract in food supplements such as tablet/capsule or sachets (e.g. tea or soup-style formulations), for delivery as a stand-alone drink or for the addition to other beverages/foodstuffs. The applicant proposes that food supplements should be consumed as a dose of 0.3 g with every meal of the day. Considering usual meal intakes of 3 - 4 per day the applicant anticipates an average intake of 0.9 - 1.2 g Touchi extract per day and proposes a maximum intake level of 4.5 g per day. The applicant indicates that adults who wish to inhibit the digestion of carbohydrates for weight management purposes are the target population. The applicant provided data, which indicate that the typical serving size of fermented black bean products contains approximately 15 g of fermented black beans. An intake estimate based on UK consumption data suggests that a daily intake of 15 g fermented black beans corresponds to the 90th percentile intake estimate for adult people in the UK. The Panel notes that intake of the corresponding proposed maximum dose of 4.5 g Touchi extract would be within the high percentile of the provided intake estimate.

The toxicological data base on the 'fermented black bean extract' is limited to data on acute toxicity, genotoxicity and a 28-day sub-chronic study in the rat, which established a NOAEL of 2500 mg/kg bw/day, the highest dose tested, equivalent to 150 g/day for a 60 kg adult. The results from the limited toxicological evaluation do not raise concerns.

The Panel notes that the clinical studies with, in total, approximately 185 subjects were primarily designed to demonstrate efficacy of the Touchi extract. The Panel notes the limitations of these studies with regard to demonstrating the human safety of Touchi extract at the proposed maximum intake levels (4.5 g/day), the study population, endpoints and documentation. However some safety parameters such as haematological and biochemical parameters and subjective side effects ("complaints") were studied and no adverse effects in subjects were reported from these studies.

The Panel considers that these studies provide limited evidence for the human safety of Touchi extract at the proposed levels.

The documentation on the safety of Touchi extract is based mainly on the history of the source of the novel food, on the significant human consumption of traditional fermented black bean products in and outside of Europe, data on the production process and on the compositional similarity between fermented black beans and Touchi extract. Toxicological and clinical data provide limited evidence for the safety of Touchi extract and do not raise concerns.

CONCLUSIONS

The Panel concludes that the 'fermented black bean extract (Touchi extract)' is safe at the proposed conditions of use.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on 19 August, 2009, submitted by CBC Co. Ltd. Additional information was submitted on 11 February, 2010 (hard copies of the dossier), 16 June 2010, 15 September 2010 and 17 February 2011.
2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety ''fermented black bean extract' '. SANCO E4/AK/bs (2009) D/540547 received on 19 August, 2009.
3. Initial assessment report provided by the competent authorities of the United Kingdom on the marketing authorization application for ''fermented black bean extract' ' as a novel food ingredient and food supplement under Regulation EC No 258/97
4. Member States' comments and objections
5. Response by the applicant to the initial assessment report and the Member States' comments and objections

REFERENCES

CBC, 2011. Additional information requested by the European Food Safety Authority (EFSA) concerning the application for approval of "Touchi Extract" as a Novel Food Ingredient under Regulation (EC) No 258/1997.

Chen YS, Yanagida F, Hsu JS, 2006. Isolation and characterisation of lactic acid bacteria from dochi (fermented black beans), a traditional fermented food in Taiwan. Letters in Applied Microbiology 43:229-235.

Commission of the European Communities. Commission Recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council (97/618/EC). *Official Journal of the European Communities* 1997, 40 (L253):1-36.

European Parliament and the Council of the European Union. Regulation EC No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. *Official Journal of the European Communities* 1997, 40 (L43):1-7.

European Parliament and the Council of the European Union. *Single Authorisation Procedure for Additives, Enzymes and Flavourings.* EUROPA, the Portal of the European Institutions, Brussels, Belgium; 2006a. Available from: http://europa.eu/scadplus/leg/en/lvb/l21304.htm.

European Parliament and the Council of the European Union. Proposal for a Regulation of the European Parliament and the Council Establishing a Common Authorisation *Procedure for Food Additives, Food Enzymes and Food Flavours*. European Commission, Brussels, Belgium; 2006b (COD 2006/0143). Available from: http://ec.europa.eu/prelex/detail_dossier_real.cfm?CL=en&DosId=194541.

Fennell DI, 1976. *Aspergillus oryzae* (NRRL Strain 1988): a clarification [With comment from Roy E. Morse]. *Science* 194:1188.

Finch S, Doyle W, Lowe C, Bates CJ, Prentice A, Smithers G and Clarke PC, 1998. National Diet and Nutrition Survey : people aged 65 years and over (NDNS 1994 – 1995). Volume 1: report of the diet nutrition survey. TSO: London.

Fujita H and Yamagami T, 2001. Fermented soybean-derived Touchi extract with anti-diabetic effect via α-glucosidase inhibitory action in a long-term administration study with KKAy mice. Life Sciences 70:219-227.

Fujita H and Yamagami T, 2007. Absence of mutagenicity, genotoxicity, and subchronic oral toxicity of Touchi extract. International Journal of Toxicology 26:465-473.

Fujita H, Yamagami T, Ohshima K, 2001a. Fermented soybean-derived water-soluble Touchi extract inhibits α-glucosidase and is antiglycemic in rats and humans after single oral treatments. Journal of Nutrition 131:1211-1213.

Fujita H, Yamagami T, Ohshima K, 2001b. Long-term ingestion of a fermented soybean-derived Touchi extract with α-glucosidase inhibitory activity is safe and effective in humans with borderline and mild Type 2 Diabetes. *Journal of Nutrition* 131(8):2105-2108.

Fujita H, Yamagami T, Ohshima K, 2003. Long-term ingestion of Touchi extract, an α-glucosidase inhibitor, by borderline and mild type-2 diabetic subjects is safe and significantly reduces blood glucose levels. *Nutrition Research* 23:713-722.

Fujita H, Yamagami T, Ohshima K, 2005. Effect of Touchi extract on blood lipids in hypertriglyceridemic subjects and Sprague-Dawley rats. Nutrition Research 25:681-692.

Hiroyuki F, Tomohide Y, Kazunori B, 2001. Efficacy and safety of Touchi extract, an α-glucosidase inhibitor derived from fermented soybeans, in non-insulin dependent diabetic mellitus. The Journal of Nutritional Biochemistry 12(6):351-356.

Hesseltine CW and Wang HI, 1980. The importance of traditional fermented foods. *Bioscience* 30(6):402-404.

Hong KJ, Lee CH, Kim SW, 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. Journal of Medicinal Food 7(4):430-435.

Hui YH, 1991. Data sourcebook for food scientists and technologists. John Wiley & Sons Chapter 2: Biotechnology pp.103-158.

Japan Food Research Laboratories, 2000a. Test of Touchi extract for Acute Oral Toxicity in Mice. Report No. 299090340-005. Japan Food Research Laboratories, Tokyo, Japan. Confidential

Japan Food Research Laboratories, 2000b. In Vitro Microbiological Mutagenicity Tests to Assess the Potential Mutagenic Effect of Touchi extract. Report No. 299090340-004. Japan Food Research Laboratories, Tokyo, Japan. Confidential

LSRO (Life Sciences Research Office), 1995. The Third Report on Nutrition Monitoring in the United States: Vols. 1&2. Federation of American Societies for Experimental Biology (FASEB), (LSRO), Interagency Board for Nutrition Monitoring and Related Research, Bethesda, Maryland

MDS Panlabs, 2000. PharmaScreen Data Report. Panlabs PT # 1004684. MDS Panlabs, King of Prussia, Pennsylvania; Confidential

Ministry of Health, Labour and Welfare of Japan, 2006. Test Methods for Soy Isoflavone Aglycones (Aglycone Equivalent) In Foods. In: Guidelines for Food for Specified Health Uses (FOSHU) etc., Including Soy Isoflavones. Appendix to Shokuanhatsu No 0823001. Ministry of Health, Labour and Welfare of Japan, Tokyo, Japan. Available from: http://www.mhlw.go.jp/houdou/2006/02/dl/h0202-1c.pdf (Japanese only)

Miwa I, Okuda J, Horie T, Nakayama M, 1986. Inhibition of intestinal α-glucosidases and sugar absorption by flavone. Chemical & Pharmaceutical Bulletin 34(2):838-844.

Murooka Y and Yamshita M, 2008. Traditional healthful fermented products of Japan. Journal of Industrial Microbiology and Biotechnology 35:791-798

Nippon Supplement Inc. Research and Development Department, 1999a. Safety Test of Touchi extract after Bolus Administration in Healthy Subjects. Nippon Supplement Inc. Research and Development Department, Osaka, Japan. Confidential

Nippon Supplement Inc. Research and Development Department, 1999b. Safety Test of Touchi extract after Long-Term Administration in Healthy Subjects. Nippon Supplement Inc. Research and Development Department, Osaka, Japan. Confidential

Office for National Statistics, 2005. The National Diet and Nutrition Survey (NDNS): Adults Aged 19 to 64 Years: 2000-2001 [Computer File]. Office for National Statistics, Social and Vital Statistics Division & Food Standards Agency (FSA), Colchester, UK. UK Data Archive [distributor]; SN: 5140.

Steinkraus, KH, 2002. Fermentations in world processing. Comprehensive reviews in food science and food safety 1; 23-32.

Stoloff L, Mislivec P, Schindler AF, 1977. *Aspergillus oryzae* (NRRL Strain 1988) [With comment from G. Morgan Jones]. Science 196:1353-1354.

UKDA (UK Data Archive), 1995. National Diet, Nutrition and Dental Survey of Children Aged 1 ½ to 4 ½ Years, 1992-1993 [computer file]. Office of Population Censuses and Surveys, Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF), and UK Department of Health. Colchester, Essex, [distributor]; SN: 3481.

UKDA (UK. Data Archive), 2001. National Diet and Nutrition Survey: Young People Aged 4 to 18 Years, 1997. Office for National Statistics Social Survey Division, Medical Research Council Centre for Human Nutrition Research, Ministry of Agriculture, Fisheries and Food (MAFF), and Department of Health. Colchester, Essex, (UKDA) [distributor]; SN: 4243.

U.S. EPA (United States Environmental Protection Agency), 1997. *Aspergillus Oryzae* Final Risk Assessment, Biotechnology Program Under Toxic Substances Control Act (TSCA), Washington, DC. Available from: http://www.epa.gov/oppt/biotech/pubs/fra/fra007.htm.

Wood BJB, 1977. Oriental Food Uses of Aspergillus. In: Smith JE, Paterman JA, eds.. Genetics and Physiology of Aspergillus. Academic Press, London, Engl, pp. 481-498 (The British Mycological Symposium Series no. 1). Available from: http://www.aspergillus.org.uk/secure/articles/Oriential%20Food%20Uses%20of%20Aspergillus.pdf.

GLOSSARY / ABBREVIATIONS

EC	European Commission
FOSHU	Food for Specific Health Use
NDA	Scientific Panel on Dietetic Products, Nutrition and Allergies
NOAEL	No observed adverse effect level
UK NDNS	UK National Dietary and Nutrition Survey
bw	body weight