

**Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies
on a request from the Commission related to the safety of
Allanblackia seed oil for use in yellow fat and cream based spreads**

(Request N° EFSA-Q-2007-059)

(Adopted on 25 October 2007 by written procedure)

SUMMARY

Allanblackia seed oil is obtained from the seeds of the *Allanblackia* tree (*A. floribunda* and *A. stuhlmannii*). The oil is isolated and refined according to commonly applied procedures. It meets the quality criteria of edible vegetable oils and the limits for potential contaminants set in the EU. Its physicochemical properties (semi-solid at room temperature) are determined by the high contents of stearic-oleic-stearic (SOS) and stearic-oleic-oleic (SOO) triglycerides (average values 69 % SOS; 23.0 % SOO). This unique arrangement of fatty acids makes the oil suitable as a hardstock component. Accordingly, *Allanblackia* seed oil is intended for use as a novel food ingredient in yellow fat and cream-based spreads.

The intake of *Allanblackia* seed oil was estimated on the basis of margarine and yellow fat spread consumption data from Germany, Sweden, the United Kingdom and the Netherlands, assuming that total margarine and yellow fat spread consumption will be replaced by products containing 20 % *Allanblackia* seed oil. Based on a 95th percentile margarine intake of 39 g/person/day across all age-groups in Germany, an intake of approximately 8 g of *Allanblackia* seed oil/person/day was estimated. On the basis of consumption data for yellow fat spreads in Sweden, the UK and The Netherlands, the applicant estimated *Allanblackia* seed oil intakes (95th percentile) to range from 2.8 g /person/day, for British boys and girls aged from 1.5 to 3.5 years, to 16 g/person/day, for Swedish women aged from 18-74 years.

Genotoxicity tests revealed no evidence of genotoxic/mutagenic properties. In a 90-day feeding study with rats, the administration of 20 % *Allanblackia* seed oil did not induce any toxicologically relevant effects which were attributable to administration of *Allanblackia* seed oil and which are not also seen in animal studies with other high fat diets.

The Panel concludes that *Allanblackia* seed oil is safe for human consumption under the specified conditions of use.

KEY WORDS

Allanblackia, seed oil, yellow fat spreads, cream-based spreads, novel food ingredient

BACKGROUND

On 30 August 2004, Unilever Deutschland GmbH, submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to the competent authorities of Germany for placing on the market '*Allanblackia* seed oil for use in yellow fat and cream-based spreads' as a novel food ingredient.

On 3 April 2006, the competent authorities of Germany forwarded to the Commission their initial assessment report, which had reached the conclusion that '*Allanblackia* seed oil for use in yellow fat and cream-based spreads' to be used as recommended by the applicant is acceptable.

On 8 June 2006, the Commission forwarded the initial assessment report to other Member States. Several of these Member States submitted additional comments/objections. The issues of a scientific nature can be summarized as follows:

- Levels of contaminants and compliance with limits set in the EU.
- It is not clear whether the samples analysed represent the full diversity of the *Allanblackia* species found in the different areas of the tropical rain forests of Africa. Only those species of *Allanblackia* used as a test material in the toxicological studies should be authorized as raw materials for the production of oil.
- Older references report that *Allanblackia parvifolia* contains an alkaloid with properties similar to physostigmine, a pharmacologically active substance.
- Due to the large difference in fat intake between the soy oil group (7 %) and the two other groups of animals (20 % *Allanblackia* oil and 20 % shea olein oil, respectively) it is difficult to conclude whether the differences found in the subchronic (90-days) oral toxicity study with rats were due to the differences in fat content of the diets or to differences between the oils. Further studies should be carried out. A developmental study merged with a 90 days study would be preferable.
- The significance of the decrease in total white blood cells and lymphocytes observed in male rats fed *Allanblackia* oil compared with the control group and shea oleine group, in the 13-week oral toxicity study was questioned.
- The safety assessment does not include tests with oil subjected to high temperatures. Therefore its use in fats for frying or for culinary processes that involve heating must be excluded from the authorisation.
- The possibility that *Allanblackia* seed oil may provoke IgE-mediated allergenicity has not been experimentally addressed. Putative seed and seed oil allergenic proteins could be extracted and estimated by SDS/PAGE immunoblotting and/or RAST/EAST assays using pooled sera from patients with food allergy and anti-IgE antibodies.
- The presence of traces of guttiferone F in the refined oil may constitute an allergy risk.
- The placing on the market of the Novel Food should be accompanied by post launch monitoring (PLM) to follow consumption and to collate information on any reported adverse effects.

In consequence, a Community Decision is now required under Article 7, paragraph 1 of Regulation (EC) N° 258/97.

TERMS OF REFERENCE

In accordance with Article 29(1) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for `Allanblackia seed oil for use in yellow fat and cream-based spreads` in the context of Regulation (EC) No 258/97.

In particular, EFSA is asked to consider the elements of scientific nature in the comments/objections raised by the other Member States.

ASSESSMENT

Allanblackia seed oil belongs to class 1, sub-category 1.2 (novel foods which are pure chemicals or simple mixtures derived from sources which have not been genetically modified and which have no history of food use within the Community), as defined in the recommendations of the Scientific Committee for Food (SCF) concerning the assessment of novel foods (European Commission, 1997b). Accordingly, information related to the structured schemes I, II, III, IX, XI, XII, XIII has been submitted.

I. Specification of the novel food (NF)

Allanblackia seed oil is obtained from the seeds of the *Allanblackia* tree. *Allanblackia* belongs to the Guttiferae family, subfamily Clusiodeae. There are several *Allanblackia* species; according to the applicant, only the species *A. floribunda* (synonymous with *A. parviflora*) and *A. stuhlmannii* are used as starting materials for seed oil production. Analytical data on fatty acid compositions provided for five refined oils from *A. floribunda* (origin: Ghana) and three refined oils from *A. stuhlmannii* (origin: Tanzania) showed no significant differences between the two species (except for an increased content of 0.27 % free fatty acids in one batch of a physically refined oil from *A. floribunda*).

The specification of refined oil derived from the seeds of *A. floribunda* or *A. stuhlmannii* as proposed by the applicant is presented in Table 1.

Table 1. Specification for refined *Allanblackia* seed oil

Lauric acid (C12:0)	< 1 %
Myristic acid (C14:0)	< 1 %
Palmitic acid (C16:0)	< 2 %
Palmitoleic acid (C16:1)	< 1 %
Stearic acid (C18:0)	45 – 58 %
Oleic acid (C18:1)	40 – 51 %
Linoleic acid (C18:2)	< 1 %
γ-Linolenic acid (C18:3)	< 1 %
Arachidic acid (C20:0)	< 1 %
Free fatty acids	max. 0.1 %

Trans fatty acids	max. 0.5 %
Peroxide value	max. 0.8 meq/kg
Iodine value	< 46 g / 100 g
Unsaponifiable matter	max. 0.1 %
Saponification value	185 – 198 mg KOH/g

Refined *Allanblackia* seed oil is semi-solid at room temperature. Its physicochemical properties are determined by the high contents of stearic-oleic-stearic (SOS) and stearic-oleic-oleic (SOO) triglycerides (average values from four batches: 69.1 % SOS; 23.0 % SOO).

As regards the presence of contaminants, the applicant has laid down the quality specification for refined *Allanblackia* seed oil as shown in Table 2. This is in accordance with the specification set by the applicant for other refined vegetable oils produced for food use and also meets the legislative limits set in the EU.

Table 2. Quality specification for refined *Allanblackia* seed oil

Property	Limit (maximum)	Method
Pesticides	n.d. ^a	AOCS Cd 23-93
Arsenic	0.1 mg/kg	ISO-11212-1
Cadmium	0.05 mg/kg	ISO-15774
Copper	0.01 mg/kg	ISO-8294
Iron	0.1 mg/kg	ISO-8294
Mercury	0.05 mg/kg	ISO-11212-2
Lead	0.1 mg/kg ^b	ISO-12193
PAH total	25 µg/kg	IUPAC 2.608
PAH heavy	5 µg/kg	IUPAC 2.608
Benzo (a) pyrene	1 µg/kg ^c	ISO-15302
Aflatoxins	n.d. ^a	
Dioxins	0.75 ng WHO-TEQ/kg ^d	

^a below the detection limits or below the maximum residue levels as defined by EU legislation

^b maximum level according to Regulation (EC) No 1881/2006

^c maximum level according to Regulation (EC) No 1881/2006: 2.0 µg/kg

^d maximum level of sum of dioxins (sum of polychlorinated dibenzo-*para*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), expressed as World Health Organisation (WHO) toxic equivalent using the WHO-toxic equivalency factors (WHO-TEFs)) according to Regulation (EC) No 1881/2006

Five batches of seed oils obtained from *A. floribunda* (origin: Ghana) and one batch obtained from *A. stuhlmannii* (origin: Tanzania) were shown to comply with the specifications for heavy metals and PAH.

Data obtained for an *Allanblackia* seed sample and two batches of refined *Allanblackia* seed oil demonstrated the levels of 18 organochlorines and of 29 organophosphates to be below

0.01 mg/kg and the levels of DDT, 4 pyrethroids and 9 organonitrogens to be below 0.05 mg/kg.

The levels of Aflatoxin B1, B2, G1 and G2, ochratoxin, zearalenone, deoxynivalenol, T-2 toxin and HT-2 toxin were below the respective limits of detection in ten batches of *Allanblackia* seed oil.

Data on levels of total dioxins and total PCBs (ng WHO-TEQ/kg) were provided for five batches of *Allanblackia* seed oil.

The unsaponifiable matter present in the oil is low (max. 0.1 %). Profiles and contents of phytosterols in twelve batches of *Allanblackia* seed oil were similar to those in other commonly consumed vegetable oils.

Allanblackia species have a history of use in the treatment of certain medical conditions in a number of African countries. Extracts of the leaves, the stem bark and the root bark are reported to be used alone or in combination with other plants to treat ailments such as upper respiratory tract infections, dysentery, diarrhoea and toothache. Earlier screenings employing paper chromatography indicated the presence of an alkaloid in *Allanblackia* showing a retention time behaviour similar to that of physostigmine (Resplandy, 1961). General compilations also refer to an alkaloid (0.01 %) in the seeds of *A. parviflora* showing resemblance in some respects to physostigmine (Hoppe, 1975; List and Hörhammer, 1967-1980). However, an unambiguous confirmation of the presence of this constituent has not been reported. In phytochemical studies of the heartwood of *A. floribunda* and the root bark of *A. stuhlmannii* compounds belonging to the benzophenone (e.g. hydrocortin, guttiferones), the xanthone (e.g. 1,3,5-trihydroxyxanthone, 4,5-dihydro-1,6,7-trihydroxy-4',4',5'-trimethoxy-furano(2,3:3,4)-xanthone) and the biflavonoid (e.g. morelloflavone, volkensiflavone) families have been identified (Locksley and Murray, 1971; Fuller et al., 1999; Nkengfack et al., 2002). These compounds are reported to exhibit a range of activities including cytotoxic, anti-inflammatory, antimicrobial and anti-fungal properties (Gil et al., 1997).

There has been no detailed and systematic compositional analysis of the seeds of *Allanblackia* species. Analytical data provided by the applicant show that guttiferone E/F initially present in some crude oil samples was removed by the subsequent filtering and refining steps to below the detection limit of 0.5 mg/kg (LC/MS) and 0.03 mg/kg (LC/MS-MS), respectively. In two samples of chemically and physically, respectively, refined *Allanblackia* seed oils the amounts of residual protein (measured as total nitrogen via combustion using the Dumas technique) were shown to be below the detection limit of the method (3 mg N/kg, corresponding to 19 mg protein/kg using a nitrogen-protein conversion factor of 6.25) and were thus consistent with values obtained for other fully refined edible vegetable oils. There are no known allergens among members of the Guttiferae to which *Allanblackia* belongs. Moreover, the applicant discusses reports demonstrating that fully refined vegetable oils rarely provoke reactions in individuals sensitised to the oilseed from which they are derived (Taylor et al., 1981; Bush et al., 1985).

II. Effect of the production process applied to the NF

The ripe fruits fallen from the *Allanblackia* trees are collected. Seeds are removed, washed and dried in the sun for several days. After inspection and packaging, the dried seeds are

transported for further processing. Isolation of the oil is achieved by pressing. Owing to the application of high pressure, a temperature rise of about 50°C may occur during this step. The crude oil is filtered, stored in heatable tanks and transported to the EU for chemical or physical refining. The chemical refining process includes initial neutralisation of the oil by addition of sodium hydroxide followed by bleaching and deodorization steps. In the course of the physical refining the oil is subjected to de-gumming and bleaching and subsequent removal of free fatty acids via deodorization. All these steps are standard procedures commonly applied in the production of edible vegetable oils. According to the applicant, a HACCP system will be in place covering the complete process.

The stability of *Allanblackia* seed oil has been assessed in an accelerated oxidative test where the oil was stored at elevated temperatures. Determination of the peroxide values demonstrated *Allanblackia* seed oil to be stable during storage at 50°C over a period of four weeks. Determinations of peroxide values, free fatty acids and acid values revealed no difference in the stability of spreads containing *Allanblackia* seed oil and spreads containing a hardstock obtained by the currently applied inter-esterification of vegetable oils.

The pattern of the fatty acids contained in the triglycerides and the degree of saturation do not give rise to the assumption that *Allanblackia* seed oil might behave differently from comparable vegetable oils when used for frying.

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

Allanblackia species grow in the tropical forests along the Gulf of Guinea and in mountainous areas of Uganda and Tanzania. The oil obtained from the seed is used locally for food preparation and soap making but has never been used on a commercial scale. In some areas the seeds are eaten by children as a high-energy snack.

IX. Anticipated intake/extent of use of the NF

Allanblackia seed oil is expected to be used in yellow fat spreads and cream-based spreads in amounts up to 20 %. The novel food ingredient will be used to produce full-fat and reduced-fat spreads.

The applicant has calculated the intake on the basis of margarine and yellow fat spread consumption data from Germany, Sweden, the United Kingdom and the Netherlands, assuming that total margarine and yellow fat spread consumption will be replaced by consumption of products containing 20 % *Allanblackia* seed oil.

On the basis of data for the consumption of margarine from the national consumption study (1985-1989) for Germany, the applicant calculated average intakes of *Allanblackia* seed oil for nine age-groups, divided according to sex; they ranged from 1.5 g/person/day, for girls and boys aged from 4-6 years, to 2.9 g/person/day, for women aged from 51-64 years. Calculations by the German competent authorities showed that the 95th percentile of margarine intake across all age-groups in Germany was 39 g/person/day, which is equivalent to approximately 8 g of *Allanblackia* seed oil per person per day.

Using data from a 12 week survey in 2002 on margarine and yellow fat spreads purchase by one-member households in Germany, the applicant estimated a mean intake of *Allanblackia* seed oil of 4 g/person/day and a 95th percentile intake of 10 g/person/day.

On the basis of the consumption data for yellow fat spreads in Sweden, the United Kingdom and The Netherlands, the applicant estimated *Allanblackia* seed oil intakes (95th percentile) to range from 2.8 g/person/day, for British boys and girls aged from 1.5 to 3.5 years, to 16 g/person/day, for Swedish women aged from 18-74 years.

Based on a daily consumption of yellow fat spreads in Europe of 20 to 30 g/person/day (SCF, 2000), an intake of *Allanblackia* oil of 4 to 6 g/person/day can be estimated. The estimated 95th percentile uses of yellow fat spreads by males above 50 years of age in the UK (57 g/day) and in The Netherlands (70 g/day) (SCF, 2000) result in intakes of *Allanblackia* oil of 11 and 14 g/person/day, respectively.

According to the applicant, complete replacement of butter (average intake of 19 g/person/day in Germany) by fat spreads with an *Allanblackia* seed oil content of 20 % would result in an average *Allanblackia* seed oil intake of 3.8 g/person/day.

The Panel notes the intention of the applicant to conduct a post launch monitoring to follow consumption of the product.

XI. Nutritional information on the NF

The fatty acids contained in *Allanblackia* seed oil are present in commonly used vegetable oils. It is intended that *Allanblackia* seed oil will replace palm oil and palm kernel oil traditionally used as hardstock components in the production of yellow fat spreads and cream-based spreads. The main differences between palm oil and *Allanblackia* seed oil are the contents in palmitic acid (32-45 % vs. < 2 %) and stearic acid (4-6 % vs. 45-58 %). Compared with palm kernel oil, the amounts of saturated fatty acids with chain lengths below C₁₆ are significantly decreased in *Allanblackia* seed oil (e.g., lauric acid 47-52 % vs. < 1 %). According to the applicant, the use of *Allanblackia* seed oil in the production of spreads would decrease the amounts of saturated fatty acids compared with currently available yellow fat spreads (from 35 g/100 g to 25.3 g/100 g in full fat formulations and from 10.9 g/100 g to 8.2 g/100 g in low fat formulations) and would increase the amounts of the monounsaturated oleic acid (from 31.8 g/100 g to 40.5 g/100 g in full fat formulations and from 14.1 g/100 g to 17.1 g/100 g in low fat formulations).

XII. Microbiological information on the NF

According to the applicant, Good Manufacturing Practice applied to the production of margarines and fat spreads will also apply to products containing *Allanblackia* seed oil. No additional controls appear necessary.

XIII. Toxicological information on the NF

Genotoxicity

In bacterial gene mutation tests using strains of *Salmonella enterica* Typhimurium TA1535, TA1537, TA98, TA100 and TA102, with and without metabolic activation (S9 mix), *Allanblackia* seed oil was not genotoxic at concentrations up to 5000 µg/plate.

In tests on gene mutations in mammalian cells (mouse lymphoma L5178Y assay) there was no evidence of a mutagenic effect, up to the highest concentration tested (1250 µg/ml), with and without metabolic activation (S9 mix).

In the *in vitro* micronucleus test, using human peripheral blood lymphocytes, the test material did not induced micronuclei in concentrations up to solubility level, with and without metabolic activation.

Subchronic toxicity

In a 13-week feeding study, groups of 20 male and 20 female Wistar rats were given feed containing 20 % *Allanblackia* seed oil or 20 % shea olein (high fat control). The oils normally contain around 53 % and 25 % stearic acid, respectively, 40 % and 46 % oleic acid, respectively, and 0.2 % and 7.5 % linoleic acid, respectively. A further control group received a normal rodent diet with a fat intake of 7 % (low fat control). According to the applicant, the low fat diet was compensated for caloric content, minerals and vitamins. However, the compositional differences in terms of fatty acids in the high fat diets remained.

The administration of 20 % *Allanblackia* oil induced numerous statistically significant differences in haematological and clinical-chemical parameters, organ weights and morphology, compared with animals receiving feed with a 7 % fat content (low fat control). However, similar effects, which were statistically significant or followed a recognisable trend, were also seen in animals, which received 20 % shea olein (high fat control). Furthermore, this reflects results reported in comparable studies following administration of high doses of other fats. Although some effects were more pronounced in animals receiving *Allanblackia* seed oil, the Panel did not identify any toxicologically relevant effects which were attributable to administration of *Allanblackia* oil and which are not also seen in animal studies with other high fat diets.

CONCLUSIONS AND RECOMMENDATIONS

The Panel concludes that *Allanblackia* seed oil is safe for human consumption under the specified conditions of use.

DOCUMENTATION PROVIDED TO EFSA

Dossier on '*Allanblackia* seed oil for use in yellow fat and cream-based spreads' as a novel food ingredient for the placing on the market as a novel food ingredient. Application pursuant to Regulation (EC) N° 258/97 submitted by Unilever Deutschland GmbH, August 2004.

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