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Safety evaluation of the food enzyme β -amylase obtained from soybean (*Glycine max*)

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Abstract

The food enzyme considered in this opinion is a β -amylase (EC 3.2.1.2) from soybean submitted by Nagase (Europa) GmbH. This β -amylase is intended to be used in the starch processing for maltose syrup production and the manufacture of a Japanese rice cake type. Based on the maximum use levels recommended for the respective food processes, dietary exposure to the food enzyme–total organic solids (TOS) was estimated on the basis of Japanese consumption data. Conservative average infant formula consumption, as reported in the EFSA Draft Guidance on risk assessment of substances present in food intended for infants below 16 weeks of age, was used to estimate the exposure to a fraction of soybean comparable to the food enzyme–TOS, resulting from the consumption of soybean-derived foods. The exposure estimate to the food enzyme–TOS was found to be lower than the comparable fraction from the source material. Potential allergenicity of the β -amylase was evaluated by searching for similarity of the amino acid sequence to those of known allergens, and no match was found. The β -amylase is produced from soybean, which is a known allergenic food. Japanese rice cake, consequently, may contain traces of soybean allergens, which may give rise to safety concerns in soybean-allergic consumers. Based on the origin of the food enzyme from edible parts of soybean, the manufacturing process, the compositional and biochemical data provided and the dietary intake estimates, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use, except that Japanese rice cake produced with this food enzyme may contain traces of soybean allergens.

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Note: The full opinion will be published in accordance with Article 5 of Regulation (EC) No 1331/2008 once the decision on confidentiality, in line with Article 12(3) of the Regulation, will be received from the European Commission. The following information has been provided under the confidentiality framework and has been redacted awaiting the decision of the Commission: steps and control points during the processing, process chemicals, yield factor of the food enzyme.

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Amendment: The term “whey” was replaced by “aqueous extract” throughout to comply with EU legislation and case law. In addition, the author name Herman was corrected. The original version and a version showing the changes made are available on request.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ provides definition for 'food enzyme' and 'food enzyme preparation':

- 'Food enzyme' means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.
- 'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances, such as food additives and/or other food ingredients, are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008² established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed,
- there is a reasonable technological need, and
- its use does not mislead the consumer.

All food enzymes currently on the EU market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on a food enzyme for evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

An application has been introduced by Nagase (Europa) GmbH for the authorisation of the food enzyme β -amylase obtained from soybean (*Glycine max*).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011³ implementing Regulation (EC) No 1331/2008, the Commission has verified that the application falls within the scope of the food enzyme Regulation and contains all the elements required under Chapter II of that Regulation.

¹ Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, p. 7–15.

² Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

³ Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.3.2011, p. 15–24.

1.1.2. Terms of Reference

The European Commission requests EFSA to perform the safety assessment on the food enzyme β -amylase obtained from soybean in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.1.2.1. Interpretation of the Terms of Reference

In the mandate from the European Commission, EFSA was requested to carry out the safety assessment of β -amylase from soybean. Evaluation of the production process made clear that this enzyme is obtained via aqueous extraction from soybean. In order to maintain consistency between the actual source material and the scope of the scientific assessment, EFSA considers soybean aqueous extract as the source of the β -amylase described in this application.

1.2. Information on existing authorisations

According to the applicant, the authorities of Japan have evaluated and authorised the use of β -amylase obtained from soybean.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme β -amylase obtained from soybean. The food enzyme is intended to be used in the production of maltose syrup and for rice cake manufacture.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009b) and following the relevant existing Guidances from the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA, 2009a) has been followed for the evaluation of this application with the exception of the exposure assessment, which was carried out in accordance to the principle described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016).

3. Assessment

3.1. Technical data

3.1.1. Identity of the food enzyme

IUBMB nomenclature:	β -amylase
Systematic name:	4- α -D-glucan maltohydrolase
Synonyms:	Saccharogen amylase, glycogenase, 1,4- α -D-glucan maltohydrolase
IUBMB No:	EC 3.2.1.2
CAS No:	9000-91-3
EINECS No:	232-566-1

The food enzyme is obtained via aqueous extraction from soybean.

3.1.2. Chemical parameters

β -Amylase obtained from the aqueous extract of soybean consists of a single polypeptide chain of 496 amino acids. The 56 kDa molecular mass was calculated from the amino acid sequence deduced from the cDNA sequence (<http://www.uniprot.org/uniprot/P10538>). The applicant provided a sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) analysis of six food enzyme batches in Table 1. Results indicate that the batch-to-batch variability is minimal. A band corresponding to the β -amylase and a more intensely stained band with a same molecular mass as the soybean lectin monomer were found in each of the six batches of product analysed.

Table 1: Compositional data provided for six commercial production batches

Parameter	Unit	Batches					
		1	2	3	4	5	6
β -Amylase activity	AUN/g batch ^(a)	32,680	34,580	35,980	35,740	37,850	34,910
Protein	mg/g	442	452	476	504	526	498
Ash	%	10.4	10.1	10.0	15.1	12.2	12.1
Water	%	8.3	8.6	8.7	8.5	8.3	8.4
Total organic solids (TOS) ^(b)	%	81.3	81.3	81.3	76.4	79.5	79.5
β -Amylase activity/mg TOS	AUN/mg TOS	40.20	42.53	44.26	46.78	47.61	43.91

(a): AUN/g batch: amyolytic unit of Nagase (see Section 3.1.3).

(b): TOS calculated as 100% - % water - % ash.

Data on chemical parameters of the food enzyme were provided for six commercial food enzyme batches. Three commercial food enzyme batches (1, 2 and 3) represent the original manufacturing process at the time of submission of the application; three other food enzyme batches (4, 5 and 6) represent a revised manufacturing process as of October 2016 (Table 1). The revised manufacturing process differs from the original process by omitting the use of [REDACTED] and [REDACTED] as process chemicals. Despite the change in the production process, the Panel considered that the batches are equivalent for the safety evaluation.

The total organic solids (TOS) content is a calculated value derived as 100% food enzyme minus % water content minus % ash. The average TOS content of all six commercial batches was 79.9% (w/w). The average enzyme activity/TOS ratio of the commercial food enzyme batches was 44.2 amyolytic unit of Nagase (AUN)/mg TOS.

All six commercial batches of food enzyme were tested for other enzyme activities (protease, α -amylase and lipase), which were below the detection limits of the employed methods.⁴

The applicant provided data for all six commercial batches demonstrating that the contents of lead, arsenic and mercury were below the limit of detection (LoD),⁵ consequently well below the limit set for lead (< 5 mg/kg) for enzymes used in food processing (FAO/WHO, 2006). The Panel considered that the measured level of cadmium and the LoD for arsenic and mercury, in the presented batches, are not of concern as they are well below the specification levels set for food additives (As: 3 mg/kg; Cd and Hg: 1 mg/kg).⁶

The food enzyme complies with the microbiological criteria as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006), which stipulate that *Escherichia coli* and *Salmonella* species are absent in 25 g of sample and total coliforms are not more than 30 colony-forming units (CFU)/g.

The applicant provided a statement that the original three batches (1, 2 and 3) of the food enzyme complies with the Maximum Residue Limits of pesticides set in the Japanese Positive List System for Agricultural Chemical Residues in Foods.⁷ The applicant provided data on the absence of mycotoxins in food enzyme batches. In addition, the applicant has put in place a quality assurance system to minimise potential contamination of the soybean aqueous extract with mycotoxins.

Antifoam agents are being used in the course of the manufacturing process. The applicant has provided information on their identity, their use levels and methods for their analysis. Taking into account the nature, the properties and the very low amounts of the antifoam agents, the manufacturing process and the quality assurance system implemented by the applicant, the Panel considers their use as of no safety concern.

The Panel considered the compositional data provided for the food enzyme as sufficient.

⁴ Limit of detection: 2 PUN (proteolytic unit of Nagase)/g for protease; 1 DUN (dexrinometric unit of Nagase)/g for α -amylase; 10 LUN (lipolytic unit of Nagase)/g for lipase.

⁵ Limits of detection for Pb: 0.05 mg/kg; As: 1 mg/kg; Cd: 0.01 mg/kg; Hg: 0.01 mg/kg.

⁶ Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364/5, 20.12.2006.

⁷ Available online at: <http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/pages/mrls-p>

3.1.3. Properties of the food enzyme

β -Amylase catalyses the hydrolysis of 1,4- α -glycosidic linkages in polysaccharides and releases successively maltose units from the non-reducing ends of the chains.

β -Amylase activity is quantified based on the measurement of released reducing sugars from hydrolysis of soluble starch and is expressed in AUN/g. The analytical principle is based on the reduction of copper (II) to copper (I). The excess of copper (II) oxidises iodide to iodine, which is titrated by thiosulfate. One AUN is defined as the amount of enzyme that will produce reducing sugars equivalent to 1 mg of glucose in 10 min and at 40°C.

The β -amylase has been characterised regarding its activity depending on temperature and pH. At pH 5.5, β -amylase is active at temperatures up to the 70°C (approximately 50% relative activity at 70°C, with an optimum of about 60°C). The pH profile has been measured within a pH range of 3.0–7.5 at 30°C (50% relative activity at pH 3.0 and about 70% relative activity at pH 7.5, with an optimum about pH 5.5). The thermostability of the β -amylase was tested over the range of 10–70°C at pH 5.5 after incubation at different temperatures for 30 min. The stability, measured under standard assay conditions, decreases rapidly above 60°C within 30 min incubation and no activity is measured after 30 min incubation at 70°C.

3.1.4. Information on the plant source material

The food enzyme is obtained from non-genetically modified soybean suitable for human consumption. The applicant provided a statement on the implementation of a quality system ensuring compliance of the raw material with Positive List System for Agricultural Chemical Residues in Food in Japan.

The aqueous extract is the filtrate obtained from acid-precipitated curd, a by-product from tofu manufacture. Soy protein ingredients play functional roles in baked foods, processed meats and other products. Applications in edible gums and detergents are also mentioned (Smith et al., 1962).

Soybeans have a long history of use in food (Erdman and Fordyce, 1989). However, soybean is also known to be a common food allergen (reviewed by Katz et al., 2014) and listed in Annex II of Regulation (EU) No 1169/2011⁸ among the substances or products causing allergies or intolerances that should be labelled.

3.1.5. Manufacturing process

A comprehensive data set related to the manufacturing process including a list of raw materials used and a flow diagram was provided. The food enzyme is manufactured in accordance with procedures based on Hazard Analysis and Critical Control Points (HACCP) principles, and following Good Manufacturing Practice (GMP).

The extraction process consists of a pretreatment and a preservation step. The absence of significant microbial contamination is analysed after the first filtration steps, before sedimentation.

In the solid/liquid separation, insoluble soybean solids are removed from the solution containing the enzyme. The food enzyme is separated from insoluble soybean materials by several filtration and purification steps.

After separation, the liquid containing the enzyme is concentrated to reach the desired enzyme activity and/or to increase the enzyme activity/TOS ratio. Concentration may be achieved by ultrafiltration, diafiltration and/or evaporation. At the end of this process, the yield of TOS from soybean aqueous extract is [REDACTED], and from soybeans the yield is [REDACTED].

The Panel considered the information provided on the raw materials and the manufacturing process as sufficient.

3.1.6. Reaction and fate in food

β -Amylase catalyses the cleavage of 1,4- α -glycosidic linkages in the starch polysaccharides amylose and amylopectin, resulting in the successive removal of maltose units from the non-reducing ends of the starch chains.

⁸ Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304, 22.11.2011, p. 18–63.

β -Amylase is specific in its action and not known to catalyse reactions other than the hydrolysis of the starch polysaccharides amylopectin and amylose. The reaction products, i.e. maltose and the remaining oligosaccharides, are naturally present in starch-containing foods.

It is reported in the dossier that β -amylase is inactivated during the processing in the rice cake production and removed from glucose syrups through the purification steps involving an ion-exchange chromatography during processing under the intended use conditions.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used in maltose syrup production⁹ and in rice cake manufacture as was provided by the applicant (Table 2).

Table 2: Intended uses and recommended use levels of the food enzyme as provided by the applicant

Food manufacturing process	Raw material	Recommended use level (mg TOS/kg RM)
Starch processing for maltose syrup production	Starch	350–1,170
Japanese rice cake manufacture	Rice flour	4–24

TOS: total organic solids; RM: raw material.

In maltose syrup production, β -amylase is added to the liquefied starch before the saccharification step, in order to convert liquefied starch into a maltose rich solution. This solution is purified and concentrated to a maltose syrup, to be used as a sweetener in a number of foodstuffs.

In the manufacture of Japanese rice cake, β -amylase is added to the processed rice flour, containing starch and sweeteners, before toasting/boiling of the cake. β -Amylase is used in order to prevent the starch retrogradation after cooling.

According to the applicant, the food enzyme is used at the minimum dosage necessary to achieve the desired reaction according to GMP.

3.2. Dietary exposure

Following the EFSA Guidance Document on food enzymes (EFSA, 2009a) a comparison was made between:

- dietary exposure to the food enzyme–TOS, resulting from the intended use as proposed by the applicant (herein referred to as 'FE–TOS'); and
- dietary exposure to a fraction of soybean comparable to the food enzyme–TOS, resulting from the consumption of soybean-derived foods (herein referred to as source material equivalent, 'SMT-Equivalent').

The Japanese consumption data reported in the FAO/WHO Chronic Individual Food Consumption Summary Statistics (CIFOSS¹⁰) database was used to estimate the exposure to the FE–TOS. The conservative average infant formula consumption, as reported in the EFSA Draft Guidance on risk assessment of substances present in food intended for infants below 16 weeks of age, was used to estimate the exposure to the SMT-Equivalence.

3.2.1. Exposure to food enzyme–TOS according to the intended use proposed by the applicant

Exposure to the FE–TOS was estimated based on the intended use and the maximum recommended use level of the food enzyme (Table 2).

Foods/ingredients derived through starch processing, i.e. maltose syrup, were excluded from the analysis. Experimental data on the significant removal (> 99%) of protein in the course of this process have been provided (documentation provided to EFSA, number 4). The Panel considered this evidence as sufficient to conclude that the presence of residual amounts of TOS after starch processing is negligible.

⁹ The description provided by the applicant has been harmonised by EFSA according to the 'EC working document describing the food processes in which food enzymes are intended to be used' - not yet published at the adoption of this opinion.

¹⁰ https://extranet.who.int/sree/Reports?op=vs&path=/WHO_HQ_Reports/G7/PROD/EXT/CIFOCOSS_Country&userid=G7_ro&password=inetsoft123

The second intended use concerns the production of the Japanese rice cake (known as 'mochi'), which requires the use of glutinous rice (*Oryza sativa* var. glutinosa) flour,¹¹ a variety that is not commonly used in the European diet. The Japanese rice cake 'mochi' is a niche product; only few products can be found on the European market.¹² No consumption records could be found in the Comprehensive Database. However, it is known that the Japanese rice cake is sold in speciality food shops or restaurants in Europe. Consequently, the Panel decided to use Japanese consumption data as an alternative indicator for exposure, since this is expected to be an over-estimation for any consumption that may arise in Europe.

Food consumption data for Japan were retrieved from the FAO/WHO CIFOCC database. This data set was considered an appropriate alternative, since the intended use is for the production of a Japanese rice cake, consumed in Japan.

Summary statistics for chronic food consumption in Japan are available in the Japanese National Health and Nutrition Survey.¹³ Different types of rice cakes (filled) are coded under the category 'traditional confectioneries'.¹⁴ The closest matching food group, namely 'sugar products and confectioneries' was retrieved from the CIFOCC database. An ingredient factor of 0.3 was applied to account for the rice flour part in a typical filled Japanese rice cake.¹⁵ Accordingly, one serving contains 30 g of mochi rice flour, equivalent to 2.5 and 0.4 g rice flour/kg body weight (bw) per serving in a 12-kg child and 70-kg adult, respectively.

Table 3 provides an overview of the derived rice flour intake from consumption of 'sugar products and confectioneries' in the Japanese population and the subsequent exposure to the FE-TOS based on the maximum intended use level of 24 mg/kg rice flour indicated by the applicant.

Table 3: Summary of estimated dietary exposure to the FE-TOS in the Japanese population

	Japanese consumption data (g/kg bw per day)		Estimated exposure to FE-TOS (mg/kg bw per day)	
	Children*	General population*	Children	General population
Min-max mean (number of surveys)	0.03–0.76 (4)	0.0–0.27 (4)	0.0–0.01 (4)	0.0–0.02 (4)
Min-max 95th percentile (number of surveys)	0.2–1.93 (4)	0.03–0.91 (4)	0.02–0.15 (4)	0.0–0.07 (4)

bw: body weight.

*: Age not specified.

3.2.2. Dietary exposure to the SMT-Equivalent

From the Japanese data, the highest exposure to the FE-TOS was found in children. The Japanese consumption data did not distinguish between the age groups of the child population. Since infants and young children are considered to be the most exposed population group (EFSA, 2011), the Panel considered the consumption of soy-based infant formula by infants as suitable comparator for intake of the SMT-Equivalent.

According to the draft scientific committee 'guidance on the risk assessment of substances present in food intended for infants',¹⁶ the recommended conservative mean intake of infant formula by an infant is approximately 200 mL/kg bw per day. To convert the intake into raw powder a conversion factor of eight¹⁷ was applied, resulting in an equivalent powder intake of 25 g/kg bw. According to Commission Directive 2006/141/EC¹⁸, infant formula is to contain a protein content of 1.8 g/100 g infant formula powder. Assuming that all protein is derived from soy hydrolysate, intake of 25 g infant formula powder/kg bw per day would result in intake of 0.45 g soy-derived protein/kg bw per day.

¹¹ <http://www.newworldencyclopedia.org/entry/Mochi>

¹² Retrieved from the Mintel GNPD Database on 1 March 2017.

¹³ http://www0.nih.go.jp/eiken/english/research/project_nhns.html

¹⁴ Outline of the National Health and Nutrition Survey Japan, 2011. Available at <http://www0.nih.go.jp/eiken/english/research/pdf/nhns2011.pdf>

¹⁵ Derived from <http://allrecipes.com/recipe/193307/easy-mochi/>

¹⁶ Version used for a publication consultation - at the time of adoption of this opinion - available at <https://www.efsa.europa.eu/en/consultations/call/170113-0>

¹⁷ As typically used by EFSA in data conversion.

¹⁸ Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. OJ L 401, 30.12.2006, p. 1–33.

Soybeans contain 36% of protein,¹⁹ therefore, the equivalent amount of soybean required to provide 0.45 g of soy protein is 1.25 g of soybean. According to the applicant, the yield of FE-TOS from the raw soy bean material is [REDACTED]%, which when applied to the 1.25 g of soy bean required to produce the required protein content in the infant formula equates to [REDACTED] mg/kg bw SMT-Equivalent (i.e. fraction of soybean) comparable to the FE-TOS.

For infant formulae, an estimation was made as follows:

- intake of 200 mL formula/kg bw per day as a conservative average level consumption value
- infant formula has 1.8 g of protein in 100 g powder
- assuming that 100% of protein source in infant formula is soy protein
- The protein content of soybeans is about 36% (USDA and McCance & Widdowson Table)

$$\begin{aligned} & [(200 \text{ mL}/8) \times 1.8\% \text{ protein content}]/36\% \text{ protein in bean} = 1.25 \text{ g soybean/kg bw} \\ & 1.25 \times [\text{REDACTED}\% \text{ TOS/soybean yield}] = [\text{REDACTED}] \text{ g/kg bw} = [\text{REDACTED}] \text{ mg SMT-Equivalent/kg bw} \end{aligned}$$

The suitability of using infant formula consumption in infants to cover the entire population was validated by assessing soy drink consumption by adults. Seven soybean-derived foods²⁰ were reported in the EFSA Comprehensive Food Consumption Database.²¹ In the case of soy drink, the highest number of consumers (88) was identified in Germany with the mean and 95th percentile consumption of 2.5 and 7.7 g/kg bw, respectively. With the exception of soybean oil, consumption data for other soybean-derived foods was scarcer and/or the percentage of soybean as an ingredient in these foods was small. Therefore, the Panel considered the intake of 2.5 and 7.7 g/kg bw soya drink as an indicator for the consumption of soybean-derived food in adults in order to assess consumption of the SMT-Equivalent.

Soya drink contains about 5.8%¹⁷ soybean. Consequently, the consumption of 2.5 and 7.7 g of soya drink is equal to 0.15 and 0.45 g of soybean. Applying the yield factor of [REDACTED]%, to the consumption of 0.15 and 0.45 g of soybean equates to the mean and 95th percentile intake of [REDACTED] and [REDACTED] mg/kg bw SMT-Equivalent comparable to the FE-TOS, respectively.

3.2.3. Comparison of the exposure estimates

Exposure to the food enzyme from consumption of the intended use (i.e. Japanese rice cake 'mochi'), calculated on the basis of FE-TOS (Table 3), was two orders of magnitude lower (approx. 3×10^2) than the exposure to the SMT-Equivalent comparable to the FE-TOS, resulting from the consumption of soybean-derived infant formula. A similar difference in magnitude (approx. 2×10^2) was seen in adults, when only soya drink was considered. The Panel considered the assessment for infants sufficient to conclude on the safety for the whole population.

3.2.4. Uncertainty analysis

Uncertainties in the exposure assessment were identified and discussed with regard to their impact on the final exposure calculation.

In accordance with the guidance provided in the EFSA Opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and are summarised in Table 4.

¹⁹ Available at <https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid>

²⁰ Soya bread, soybean (*Glycine max*), soya drink, soy sauce, soybean oil, textured soy protein, soya yoghurt.

²¹ Available at <http://www.efsa.europa.eu/en/food-consumption/comprehensiveglucaatabase>

Table 4: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction of impact	
	Exposure to FE-TOS	Exposure to comparable SMT-Equivalent
Model input data		
Use of Japanese consumption data as indicator for European population exposure to a traditional Japanese food	+	NA
Japanese consumption data: representativeness/underreporting/misreporting/no portion size standard	+/-	NA
Use of the food category 'sugar products and confectionaries'	+	NA
Different source of food consumption data for the estimation of the FE-TOS and the SMT-equivalent	+/-	+/-
Use of intake recommendation for infant formula	NA	+
Model assumptions and factors		
Food categories included in the exposure assessment were assumed to always contain the FE-TOS	+	NA
Exposure to FE-TOS was calculated based on the maximum recommended use level	+	NA
Use of recipe fractions in disaggregating the rice cake and soy drink	+/-	+/-
Extrapolation from infant exposure to entire population	NA	+
Assuming that all protein in infant formula is derived from soy protein	NA	+
Use of legal recommendation to derive the protein content of soy-based infant milk	NA	+/-
Percent protein in soybean was assumed to be 36%	NA	+/-

+: uncertainty with potential to cause overestimation of exposure; -: uncertainty with potential to cause underestimation of exposure; NA: not applicable.

Different sources of food consumption data were used in deriving exposure estimates to FE-TOS and the SMT-equivalent, and should not be compared directly. However, the observed levels provide an indication for the difference in level of exposure from either the FE-TOS or the SMT-Equivalent.

The approach applied to the exposure estimate to food enzyme-TOS is likely to have led to an overestimation of the exposure applicable to the European population. For the estimation of intake of the SMT-Equivalent, uncertainties taken into account do not indicate either over- or underestimation of intake.

Overall, the Panel noted that the impact of the various uncertainties identified, on the estimated exposure levels derived for the purposes of establishing evidence of safe history of use are small.

3.3. Toxicological data

According to the Commission Implementing Regulation (EU) No 562/2012²², an application for the safety evaluation of a food enzyme does not need to include toxicological data if the food enzyme is obtained from edible parts of a plant intended or reasonably expected to be ingested by humans.

According to the EFSA Guidance on the submission of a dossier on food enzymes for safety evaluation, the justification for not supplying toxicological data may include a documented history on the safety of the source of the food enzyme, the composition and the properties of the food enzyme, as well as its use in foods, demonstrating no adverse effects on human health when consumed in a comparable way (EFSA, 2009a).

The Panel considers these requirements as being fulfilled because:

- 1) soybean and soy products are routinely eaten in many parts of the world (Erdman and Fordyce, 1989).

²² Commission Implementing Regulation (EU) No 562/2012 of 27 June 2012 amending Commission Regulation (EU) No 234/2011 with regard to specific data required for risk assessment of food enzymes. OJ L 168, 28.6.2012, p. 21–23.

- 2) soybean used for the production of the food enzyme complies with the legal requirements regarding limits of potential contaminants and residues in the country of manufacture. The manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns.
- 3) the compositional data provided on the food enzyme are considered sufficient.

Exposure to the food enzyme, calculated on the basis of food enzyme-TOS, is lower than the exposure to the SMT-Equivalent.

3.4. Allergenicity

Potential allergenicity of β -amylase from soybean was assessed by comparing the amino acid sequence deduced from the cDNA sequence (<http://www.uniprot.org/uniprot/P10538>) with those of known allergens according to the EFSA Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion; no matches were found.

Soybean β -amylase is not described as a potential allergen and no food allergic reactions to soybean β -amylase have been reported, so there is no evidence for potential allergenicity of the β -amylase from soybean. However, the food enzyme is derived from soybean, i.e. one of the substances or products causing allergies or intolerances⁸ and therefore the enzyme might contain traces of soybean allergens (reviewed by Batista et al., 2007).

The sugar syrups produced with the food enzyme are not considered to be of concern regarding allergenicity given the absence of exposure as the food enzyme is removed during processing. The only food to be treated with soybean β -amylase, which may contain traces of soybean, is Japanese rice cake, which may give rise to safety concerns for soybean-allergic consumers.

Overall, the Panel considered that for sugar syrups produced with this food enzyme, there is no concern with respect to allergenicity, but that Japanese rice cake produced with this food enzyme may contain traces of soybean allergens.

Conclusions

Based on the manufacturing process and the compositional and biochemical data provided, and taking into account the dietary intake assessment, the Panel considered the food enzyme β -amylase obtained from soybean not to give rise to safety concerns under the intended conditions of use, except that Japanese rice cake produced with this food enzyme may contain traces of soybean allergens.

Documentation provided to EFSA

- 1) Dossier 'Request for the authorization of a β -amylase preparation from *Glycine max* for use as a food processing aid'. March 2015. Submitted by Nagase (Europa) GmbH.
- 2) Additional information received from Nagase (Europa) GmbH in October 2016 regarding protein analysis, process chemicals, typing errors, raw material and yield.
- 3) Additional information received from Nagase (Europa) GmbH in January 2017 regarding certificates of analysis, analysis methods for heavy metals, protein content, quality assurance systems, antifoams, protein analysis and confidentiality.
- 4) Additional information on 'Food enzyme removal during the production of cereal based distilled alcoholic beverages' and 'Food enzyme carry-over in glucose syrups'. 22 February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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Abbreviations

AUN	amylolytic unit of Nagase
Bw	body weight
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU	colony-forming units
CIFOCOss	Chronic Individual Food Consumption Summary Statistics
DUN	dextrinometric unit of Nagase
EC	Enzyme Commission
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
LUN	lipolytic unit of Nagase
PUN	proteolytic unit of Nagase
SDS-PAGE	sodium dodecyl sulfate-poly acrylamide gel electrophoresis
SMT	source material
TOS	total organic solids
WHO	World Health Organization