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Safety evaluation of the food enzyme L-ascorbate oxidase from *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne

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Abstract

The food enzyme L-ascorbate: oxygen oxidoreductase (EC 1.10.3.3) is extracted from fruit peels of *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne by Nagase (Europa) GmbH. This enzyme is intended to be used in baking and cereal-based processes. Based on maximum use levels recommended for the respective food processes and individual data from the EFSA Comprehensive European Food Consumption Database, dietary exposure to the food enzyme–total organic solids (TOS) was estimated to be up to 5.950 mg TOS/kg body weight per day in European populations. This exposure is in the same order of magnitude for infants and toddlers; but for children, adolescents, adults and the elderly it is one order of magnitude higher than the exposure to the fraction of the fruit peels comparable to the food enzyme–TOS. The Panel, while recognising the order of magnitude of difference in the exposure estimates, considers that any realistic exposure derived from the use of the food enzyme would be considerably lower and likely to be within the range of exposure through a typical diet. The Panel agreed that the requirements for exclusion of toxicological data were met. Amino acid sequence similarity to known allergens was searched and no match was found. The Panel considered that the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but are not expected to exceed the likelihood of the allergic reactions following consumption of pumpkin or zucchini per se, which is low. Based on the data provided and the origin of the food enzyme from edible parts of *C. pepo* L. and *C. moschata* Duchesne, the Panel considers that the food enzyme L-ascorbate oxidase does not raise safety concerns under the intended conditions of use.

Keywords: food enzyme, L-ascorbate oxidase, EC 1.10.3.3, peel, *Cucurbita pepo* L., *Cucurbita moschata* Duchesne

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definitions for 'food enzyme' and 'food enzyme preparation'.

> 'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (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 1331/2008[2] established 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:

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

All food enzymes currently on the European Union 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 an approval via a Union list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA CEF Panel, 2009) 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 European Union (EU) Community 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.

Five applications have been introduced by the companies "Chr.Hansen" for the authorisation of the food enzyme Endothiapepsin from a genetically modified strain of *Cryphonectria parasitica* (strain DSM 29549), "Nagase (Europa) GmbH" for the authorisation of the food enzymes L-ascorbate oxidase from *Cucurbita pepo* and *Cucurbita moschata*, and Microbial collagenase from a genetically modified strain of *Streptomyces violaceoruber* (strain pCol); "Novozymes A/S" for the authorisation of the food enzyme Inulinase from *Aspergillus niger* (strain NZYM-KF), and "Danisco US Inc." for the authorisation of the food enzyme Endo-1,3,(4)-beta-glucanase from a genetically modified strain of *Bacillus subtilis* (DP-Ezm28).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the five applications fall within the scope of the food enzyme Regulation and contain 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, pp. 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 the European Food Safety Authority to carry out the safety assessment of the food enzymes Endothiapepsin from a genetically modified strain of *Cryphonectria parasitica* (strain DSM 29549), L-ascorbate oxidase from *Cucurbita pepo* and *Cucurbita moschata*, Microbial collagenase from a genetically modified strain of *Streptomyces violaceoruber* (strain pCol); Inulinase from *Aspergillus niger* (strain NZYM-KF) and Endo-1,3,(4)-beta-glucanase from a genetically modified strain of *Bacillus subtilis* (DP-Ezm28) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Interpretation of the Terms of Reference

The present scientific opinion addresses the European Commission request to carry out the safety assessment of food enzyme L-ascorbate oxidase from *Cucurbita pepo* L. and *Cucurbita moschata* Duchesne.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme L-ascorbate oxidase obtained from *C. pepo* and *C. moschata*. It was updated on 23 November 2017, 23 March 2019, 12 April 2019 and 16 April 2019.

Additional information was requested from the applicant during the assessment phase on 29 September 2017, 20 December 2017, 5 December 2018 and 28 March 2019, and was consequently provided (see 'Documentation provided to EFSA').

Following the request by the WG, a technical hearing was held with the applicant on 22 November 2018.[4]

Following the reception of additional information from the applicant on 26 February 2019, EFSA requested a clarification teleconference, which was held on 28 March 2019.

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, 2009) and following the relevant existing guidance's of EFSA Scientific Committees.

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

3. Assessment

IUBMB nomenclature:	L-ascorbate oxidase
Systematic name:	L-ascorbate: oxygen oxidoreductase
Synonyms:	AA oxidase; ascorbase; L-ascorbic acid oxidase; ascorbate dehydrogenase
IUBMB No:	EC 1.10.3.3
CAS No:	9029-44-1
EINECS No:	232-852-6

L-Ascorbate oxidase belongs to the family of oxidoreductases acting on diphenols and related substances as donor with oxygen as acceptor. It catalyses the oxidation of L-ascorbate with oxygen to dehydroascorbate with the generation of water. The food enzyme is intended to be used in baking processes and cereal-based processes.[5]

3.1. Source of the food enzyme

The food enzyme L-ascorbate oxidase is obtained from the fruit peels of two non-genetically modified Cucurbitaceae species – *Cucurbita pepo* L. (from here on referred to as *C. pepo*) and/or

[4] https://www.efsa.europa.eu/sites/default/files/wgs/food-ingredients-and-packaging/enzymes-min.pdf
[5] See Appendix A for a list of possible food products.

Cucurbita moschata Duchesne (from here on referred to as *C. moschata*) - depending on seasonal availability.[6]

Cucurbita are vines native to Central and North America, but are now cultivated throughout the world and rank among the ten most important vegetable crops grown. *C. pepo* and *C. moschata* are both annual plants and are consumed throughout the year. There are many common names recognised for the fruits of the various varieties/cultivars including, e.g. for *C. pepo*, pumpkin, squash and zucchini/courgette. The term squash is also widely applied to the fruits of *C. moschata,* as in butternut squash (Schaffer and Paris, 2003). All aerial parts of the plants are considered edible including flowers and young stems in addition to the fruit.[7]

The only known toxins produced by *Cucurbita* spp. are a group of cytotoxic steroids referred to as cucurbitacins. These have a strong bitter taste and for this reason are absent or found only in very low levels in varieties selected for human consumption (Hsu et al., 2014).

3.2. Production of the food enzyme

The food enzyme is manufactured in accordance with Good Manufacturing Practice and under quality assurance certification (ISO 9001). The production process complies with the Japanese Food Sanitation Law for food additives, which also requires implementation of a Hazard Analysis and Critical Control Point plan and is considered equivalent to the European standard and legislation.[8]

The food enzyme is obtained by extraction with water during a grinding process and subsequent separation from the insoluble material by centrifugation and filtration. After separation, the liquid containing the enzyme is concentrated by ultrafiltration, then polished by passing through diatomaceous earth and finally through a membrane filter to ensure that microbial contamination is minimal. After this stage, the enzyme concentrate is blended with dextrin to provide a consistent product, freeze-dried, ground and packaged. At the end of this process, 1 kg of the food enzyme concentrate (excluding dextrin) can be obtained from [redacted] of fruit peels on average, which corresponds to a yield factor of [redacted] (w/w).[9]

The applicant has provided information on the identity of the substances used in the extraction and in the subsequent processing of the food enzyme.[10]

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The L-ascorbate oxidase freshly extracted from *C. pepo* is a heterodimer with a molecular mass of 145 kDa with subunits of 75 and 72 kDa. After incubation with sodium dodecyl sulfate and reducing agents at neutral pH, it migrated to a position corresponding to 68 kDa on a polyacrylamide gel (Avigliano et al., 1983). Under the same experimental conditions, three commercial batches of the food enzyme concentrate obtained from the *C. moschata* also migrated to a position corresponding to 68 kDa on the polyacrylamide gel.[11]

The full amino acid sequence of the enzyme from *C. pepo* together with its crystal structure is available from the National Center for Biotechnology Information (NCBI), US National Library of Medicine database.[12] For the L-ascorbate oxidase from *C. moschata*, the nucleotide sequence is available at the NCBI (accession number E03088[13]), which is identical to the full-length cDNA sequence of the ascorbate oxidase isolated from cultured cells of the *Cucurbita* sp. Ebisu Nankin (Esaka et al., 1990).[14] From this information, the applicant generated the amino acid sequence using the ExPASy translation tool and the estimated molecular mass is about 61 kDa. Comparison of the two

[6] Technical dossier/Section 3.3.4.3 and Additional data December 2017.
[7] Technical dossier/Additional data February 2019.
[8] Technical dossier/Additional data December 2017.
[9] Technical dossier/Additional data April 2019.
[10] Technical dossier/Section 3.4.1.
[11] Technical dossier/p. 23.
[12] https://www.ncbi.nlm.nih.gov/protein/1ASP_A.
[13] https://www.ncbi.nlm.nih.gov/nuccore/E03088.
[14] Technical dossier/p. 24.

sequences showed that there was little difference between the food enzyme from the two sources (identity 97%, similarity 99%).[15]

The food enzyme concentrate was tested for amylase, protease and lipase activities. Amylase was detected in all three of the batches shown in Table 1 at a mean activity of 9.6 U/g total organic solids (TOS), while protease and lipase activities were below the respective limits of quantitation (LoQ) of the methods used.[16,17] No other enzymatic side activities were reported.

The in-house determination of L-ascorbate oxidase activity is based on the oxidation of the substrate L-ascorbic acid (reaction condition: 30°C, 5 min). The enzymatic activity is determined by measuring spectrophotometrically at 245 nm the degradation of ascorbic acid after acidification to stop the reaction. One unit of activity is defined as the amount of enzyme required to decrease the concentration of ascorbic acid by one μmol/min.[18]

The L-ascorbate oxidase has a pH optimum between pH 6 and 8 (30°C) and a temperature optimum of 30–40°C (pH 5.6). Thermostability was tested after a pre-incubation of the food enzyme for 10 min at different temperatures. Under the conditions of the applied temperature stability assay, enzyme activity is retained up to 35°C, but decreased rapidly at 50°C and the activity was lost at 70°C.[19]

3.3.2. Chemical parameters

Data on chemical parameters of the food enzyme preparation were provided for ten commercial batches (Table 1). The botanical source of these batches was not identified but this is not considered necessary given the similarity of the food enzymes derived from the two species. The mean TOS was 4.47%. The mean enzyme activity/TOS ratio was 153.3 U/mg TOS.

Table 1: Compositional data from ten batches of the food enzyme preparation[9]

Parameter	Unit	Mean	Minimum-Maximum
L-Ascorbate oxidase activity	U/g batch[(a)]	5,531	4,730–6,450
Protein	%	0.340	0.150–0.534
Ash	%	4.69	3.55–6.65
Water	%	3.34	2.43–4.99
Dextrin (excipient)	%	87.5	86.0–90.5
TOS[(b)]	%	4.47	1.97–7.02
L-Ascorbate oxidase activity/mg TOS	U/mg TOS	153.3	71.9–311.6

(a): U: L-ascorbate oxidase units.
(b): TOS calculated as 100% - % water - % ash - % excipient.

3.3.3. Purity

The lead content was below the limit of detection (LoD)[20] in all of the 10 commercial batches shown in Table 1, which complies with the specification for lead (< 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the level of arsenic was below the LoD of the employed method.[21] For cadmium and mercury, the mean concentrations determined in the commercial batches were 0.06 and 0.02 mg/kg, respectively.[22] These concentrations are generally below or in line with maximum levels set for commonly consumed foodstuffs, and therefore, are not of safety concern.

The food enzyme concentrate 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 should not exceed 30 colony forming units (CFU) per gram. Additionally, total viable bacteria were found at an average of 100 CFU/g. Sulfite-reducing anaerobes and *Staphylococcus aureus* could not be

[15] Technical dossier/p. 25.
[16] Technical dossier/p. 30.
[17] LoQ: protease = 2 PUN/g TOS; Amylase = 1 DUN/g TOS; Lipase = LUN/g TOS.
[18] Technical dossier/p. 26 and Annex A2.6.
[19] Technical dossier/p. 29.
[20] LoD: Pb = 0.05 mg/kg.
[21] LoD: As (as As_2O_3) = 1 mg/kg.
[22] LoD: Cd = 0.01 mg/kg; Hg = 0.01 mg/kg.

detected in 1 g product. No antimicrobial activity was detected in any of these batches (FAO/WHO, 2006).[23]

Residue data for an extensive list of pesticides were provided for three batches; results were always below the respective limit of detection.[24]

3.4. Toxicological data

3.4.1. Toxicology

According to the Commission Implementing Regulation (EU) No 562/2012[25], 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 CEF Panel, 2009).

The Panel considers that these requirements are fulfilled, because:

i) Cucurbitaceae fruit, including peels, are commonly consumed throughout the world as vegetables, including in all European countries;
ii) the manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns. Contaminants and pesticides residues that could be carried over from the peels of *C. pepo* and *C. moschata* were analysed and raised no issues;
iii) the compositional data provided on the food enzyme are considered sufficient.

3.4.2. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of L-ascorbate oxidase from both *C. pepo* and *C. moschata* was assessed by comparing their amino acid sequences with those of known food allergens according to the Guidance on allergenicity assessment of genetically modified plants (EFSA GMO Panel, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as criterion, no matches were found.[26]

The food enzyme L-ascorbate oxidase from *C. pepo* or *C. moschata*, is not listed as an allergen in allergen data bases (the IUIS allergen data base and the AllergenOnline database) and no allergic reactions have been reported in workers exposed to the L-ascorbate oxidase preparation. However, though instances are rare, several case reports have been published dealing with food allergic reactions to pumpkin seeds (Patel and Bahna, 2016; Patel and Abdur, 2017) or to cooked pumpkin (Figueredo et al., 2000; Hagendorens et al., 2009) or zucchini soups (Reindl et al., 2000).

The allergens of pumpkin seed have not been well characterised. Both a 1- kDa protein (Fritsch et al., 1997), which is probably a homologue of profilin, and a heat-stable lipid transfer protein with molecular weight 12 kDa are proposed as allergens (Rodrıguez-Jimenez et al., 2010). Clinical cross-reactivity may occur between pumpkin seeds and members of the Rosaceae family (Gonzalez De Olano et al., 2010; La Shell et al., 2010). Food allergy to zucchini (*C. pepo* var. *cylindrica*) can occur as a result of primary sensitisation to zucchini, as well as to cross-reactions to the pan allergen profilin and cross-reacting carbohydrate determinants (Reindl et al., 2000).[27]

Considering the extraction and processing of the food enzyme concentrate, the enzyme L-ascorbate oxidase from both *C. pepo* and *C. moschata* might contain traces of allergens from pumpkin seeds or pumpkin pulp. The Panel considers that allergic reactions to this L-ascorbate oxidase from both *C. pepo* and *C. moschata* can therefore not be excluded in individuals allergic to these plants. However, the likelihood of allergic reaction to the enzyme L-ascorbate oxidase from both *C. pepo* and *C. moschata* is expected not to exceed the likelihood of allergic reactions to pumpkin or zucchini. As prevalence of

[23] Technical dossier/p. 44 and Additional data April 2019.
[24] Technical dossier/Additional data December 2017/Annex 2.5.
[25] 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.
[26] Technical dossier/Additional Information December 2017/Annex Q6(2).
[27] Technical dossier/Additional Information December 2017/Annex Q6(1).

allergic reaction to these foods is infrequent, the likelihood of such reaction to occur to the food enzyme is also considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in baking processes and cereal-based processes[28] at the maximum recommended use level of 0.5 g TOS/kg flour.[29]

The food enzyme is added to flour during the preparation of dough. L-Ascorbate oxidase acts in conjunction with the glutathione dehydrogenase already present in the dough. The food enzyme first oxidises ascorbic acid to dehydroascorbic acid. Then, glutathione dehydrogenase uses the dehydroascorbic acid as an electron acceptor in the oxidation of the reduced glutathione present in the flour (Walther and Grosch, 1987). These enzymatic reactions result in decreased levels of reduced glutathione. Consequently, the technological effect is the maintained or increased strength of the gluten structure in the dough.

The food enzyme remains in the final foods. Based on data provided on thermostability (see Section 3.3.1), it is anticipated that the L-ascorbate oxidase is inactivated by heat during baking and cereal-based processes.

3.5.2. Dietary exposure estimation

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

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

In both cases, chronic exposure was calculated using the methodology described in the CEF Panel 'Statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016). The assessment involved selection of relevant food categories from the EFSA Comprehensive European Food Consumption Database[30] and application of process and technical conversion factors (Annex B in EFSA CEF Panel, 2016).

Chronic exposure was calculated by combining the maximum recommended use level provided by the applicant (see Table 2) with the relevant FoodEx categories (Annex B in EFSA CEF Panel, 2016), based on individual consumption data. Exposure from all FoodEx categories was subsequently summed up, averaged over the total survey period and normalised for body weight. This was done for all individuals across all surveys, resulting in distributions of individual mean exposure. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per age class. Surveys with only 1 day per subject were excluded and high-level exposure/intake was calculated for only those population groups in which the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011).

Table 2 provides an overview of the derived exposure estimates across all surveys. Detailed mean and 95th percentile exposure to the FE–TOS per age class, country and survey, as well as contribution from each FoodEx category to the total dietary exposure are reported in Appendix A – Tables 1 and 2. For the present assessment, food consumption data were available from 35 different dietary surveys (covering infants, toddlers, children, adolescents, adults and the elderly), carried out in 22 European countries (Appendix B).

[28] 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 time of adoption of this opinion.
[29] Technical dossier/p. 47.
[30] Available from: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

Table 2: Summary of estimated dietary exposure to the FE–TOS in six population groups

Population group	Estimated exposure (mg/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.381–1.737 (10)	1.466–3.706 (14)	1.764–3.227 (19)	0.921–2.001 (18)	0.610–1.343 (19)	0.550–1.264 (18)
Min–max 95th percentile (number of surveys)	1.663–5.950 (8)	3.363–5.265 (12)	2.961–5.880 (19)	1.677–3.906 (17)	1.267–2.334 (19)	1.037–2.147 (18)

The chronic dietary exposure to the SMT–Equivalent was calculated by first estimating the intake of *Cucurbita* spp. from all dietary sources (applying recipe and conversion fractions reported in Appendix C). Secondly, the so-derived intake of *Cucurbita* spp. was converted by applying a factor of [redacted][31] to derive the corresponding amount of fruit peels. Finally, intake was further converted into a fraction comparable to the food enzyme–TOS via application of a yield factor provided by the applicant to take into account the yield of the FE–TOS from the fruit peel of the *Cucurbita* spp. (Section 3.2).

As many cultivars of these two *Cucurbita* species are edible and known to be consumed in the EU, the selection of food groups was aided by common names reported in the original national dietary surveys, i.e. courgettes (zucchini) and pumpkins (Appendix C). Seed(s) and seed oil from these vegetables were excluded. A small number of composite foods which potentially contain pumpkin or courgette as an ingredient were also excluded (Appendix D), due to the absence of detailed information on the ingredients used. This exclusion is unlikely to greatly influence the exposure estimates.

Table 3 provides an overview of the estimated exposure to the SMT–Equivalent. Mean and 95th percentile exposure to the SMT–Equivalent per age class, country and survey are reported in Appendix A– Table 3. The contribution of the SMT–Equivalent from each FoodEx category to the total dietary exposure is indicated in Appendix A – Table 4.

Table 3: Summary of estimated dietary exposure to the SMT–Equivalent, resulting from the consumption of *Cucurbita* spp. and *Cucurbita* spp.-derived foods, in six population groups

Population group	Estimated exposure (mg/kg body weight per day)					
	Infants	**Toddlers**	**Children**	**Adolescents**	**Adults**	**The elderly**
Age range	3–11 months	12–35 months	3–9 years	10–17 years	18–64 years	≥ 65 years
Min–max mean (number of surveys)	0.002–0.669 (10)	0.002–0.503 (14)	0.000–0.176 (19)	0.000–0.050 (18)	0.000–0.056 (19)	0.001–0.057 (18)
Min–max 95th percentile (number of surveys)	0.008–1.541 (8)	0.000–1.263 (12)	0.000–0.508 (19)	0.000–0.263 (17)	0.000–0.276 (19)	0.000–0.267 (18)

Exposure to the FE–TOS (Table 2) is in the same order of magnitude for infant and toddlers, but for children, adolescents, adults and the elderly it is one order of magnitude higher than the exposure to the SMT–Equivalent (Table 3).

3.5.3. Uncertainty analysis

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.

[31] Technical dossier/Additional data April 2019/Annex 2b.

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

Sources of uncertainty	Direction of impact	
	Exposure to FE–TOS	Exposure to SMT–Equivalent
Model input data		
Consumption data: different methodologies/representativeness/ underreporting/misreporting/no portion size standard	+/–	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+	+
Possible national differences in categorisation and classification of food	+/–	+/–
Model assumptions and factors		
FoodEx categories included in the exposure assessment were assumed to always contain the FE–TOS	+	NA
Exposure to FE–TOS was always calculated based on the maximum recommended use level	+	NA
Selection of broad FoodEx categories for the exposure assessment	+	NA
Use of recipe fractions in disaggregation FoodEx categories	+/–	+/–
Use of technical factors in the exposure model	+/–	+/–
Selection of FoodEx categories likely to contain *Cucurbita* spp. for the intake assessment of SMT–equivalent, based on the national food descriptors	NA	+/–
Exclusion of composite foods that contain Cucurbitaceae as ingredients	NA	–
The applied yield factor was the mean value from ten commercial batches	NA	+/–

+: uncertainty with potential to cause overestimation of exposure; –: uncertainty with potential to cause underestimation of exposure; NA: not applicable; TOS: total organic solids; FE: food enzyme; SMT: source material.

Both estimates are derived using the same food consumption data and exposure model, and hence share a number of uncertainties, which do not have an effect on the comparison of the two estimates.

The conservative approach applied to the exposure estimate to FE–TOS assumptions made on the use of this specific food enzyme, which is a product of limited technological use, is likely to have led to a considerable overestimation of the exposure. Consequently, the Panel, while recognising the order of magnitude of difference in the exposure estimates, considers that any realistic exposure derived from the use of the food enzyme would be considerably lower and likely to be within the range of exposure through a typical diet.

Conclusions

Based on the data provided and the origin of the food enzyme from edible parts of *C. pepo* L. and *C. moschata* Duchesne, the Panel considers that the food enzyme L-ascorbate oxidase does not raise safety concerns under the intended conditions of use.

Documentation provided to EFSA

1) Dossier 'Request for the authorisation of a L-ascorbate oxidase preparation from *Cucurbita pepo* and *Cucurbita moschata* for use as a food processing aid'. February 2014. Submitted by Nagase (Europa) GmbH and updated in November 2017, March 2019 and April 2019.
2) Additional information. December 2017. Submitted by Nagase (Europa) GmbH.
3) Additional information. February 2018. Submitted by Nagase (Europa) GmbH.
4) Additional information. February 2019. Submitted by Nagase (Europa) GmbH.
5) Additional information. April 2019. Submitted by Nagase (Europa) GmbH.

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Abbreviations

CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
DUN	dextrinogenic unit
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
FE–TOS	Food enzyme–TOS

IUBMB	International Union of Biochemistry and Molecular Biology
LoD	limit of detection
LoQ	limit of quantitation
NCBI	National Center for Biotechnology Information
PUN	proteolytic unit
SMT–Equivalent	source material TOS equivalent
TOS	total organic solids
U	L-ascorbate oxidase unit
WHO	World Health Organization

Appendix A – Dietary exposure estimates to the food enzyme–TOS and the SMT–Equivalent in details

Information provided in this appendix is shown in an excel file (downloadable https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.5740).

The file contains four sheets, corresponding to four tables

Table 1: Mean and 95th percentile exposure to the FE-TOS per age class, country and survey

Table 2: Contribution of FoodEx categories to the FE-TOS dietary exposure

Table 3: Mean and 95th percentile exposure to the SMT–Equivalent per age class, country and survey

Table 4: Contribution of FoodEx categories to the SMT–Equivalent dietary exposure

Appendix B – Population groups considered for the exposure assessment

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks on up to and including 11 months of age	Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Portugal, United Kingdom
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, United Kingdom
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Netherlands, Portugal, Spain, Sweden, United Kingdom
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Portugal, Romania, Spain, Sweden, United Kingdom

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011).

Appendix C – FoodEx categories used to derive intake estimates of SMT–Equivalent and the respective conversion factors

FoodEx code[(a)]	FoodEx name[(a)]	FAO conversion factor from FoodEx food group to *Cucurbita* fruit	Proportion of peels in *Cucurbita* fruit[(b)]
A.02.03.008	Courgettes (Zucchini) (*Cucurbita pepo* var. *melopepo*)	1	[redacted]
A.02.03.010	Pumpkins (*Cucurbita maxima*)	1	[redacted]

SMT–Equivalent: source material TOS equivalent; TOS: total organic solid.
(a): Only if *Cucurbita* (zucchini, pumpkin, squash, vegetable marrow) is clearly indicated in the original food name, surveys were included in the calculation.
(b): Additional data April 2019.

Appendix D – FoodEx categories excluded from intake estimates of SMT–Equivalent

FoodEx code	FoodEx name	Conversion factor from FoodEx food group to *Cucurbita* fruit[(a)]	Proportion of peels in *Cucurbita* fruit[(b)]
A.19.01.003.001	Pasta, cooked, with vegetables	0.0357	[redacted]
A.19.01.003.005	Pasta, cooked, vegetable filling	0.0436	[redacted]
A.19.02.001	Rice and vegetables meal	0.085	[redacted]
A.19.07.001	Mixed vegetables, grilled	0.20	[redacted]
A.19.07.007	Ratatouille	0.0933	[redacted]
A.19.10.008	Mushroom soup	0.102	[redacted]
I.19.07.005.003	Vegetarian moussaka	0.0543	[redacted]
I.19.03.002.006	Pytti i panna	0.0401	[redacted]

SMT–Equivalent: source material TOS equivalent; TOS: total organic solid.
(a): Source: the EFSA raw primary commodity (RPC) model, available at https://doi.org/10.5281/zenodo.2537955
(b): Additional data April 2019.