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Safety evaluation of the food enzyme β -amylase obtained from barley (*Hordeum vulgare*)

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Abstract

The food enzyme considered in this opinion is a 4- α -D-glucan maltohydrolase (EC 3.2.1.2) obtained from grain of barley (*Hordeum vulgare*), by the companies Genencor International B.V. and Senson Oy. This β -amylase is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups. The compositional data provided for the food enzyme were considered sufficient. The manufacturing process did not raise safety concerns. 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 individual data from the EFSA Comprehensive European Food Consumption Database. This exposure estimate is similar to or lower than the exposure to a fraction of barley comparable to the food enzyme–TOS, resulting from the consumption of barley-derived foods. As the food enzyme is derived from edible parts of barley, in line with the requirements of the guidance document on food enzyme assessment, the Panel accepted that there was no need for the provision of toxicological data for this food enzyme. Barley is known as a gluten-containing cereal; however, the gluten content in the food enzyme was found to be below the detection limit of the applied analytical method and well below the threshold value of 20 mg/kg for 'gluten-free' products. Furthermore, the potential allergenicity was evaluated by searching for similarity between the amino acid sequence of β -amylase and the sequences of known food allergens; no match was found. Based on the origin of the food enzyme from edible parts of barley, the enzyme-manufacturing process, the compositional and biochemical data provided, the allergenicity and dietary exposure assessment, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ 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 1331/2008² 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,
- 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, 2009b) 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 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. According to this Regulation, a food enzyme that falls within the scope of Regulation (EC) No 1829/2003³ on genetically modified food and feed should be authorised in accordance with that Regulation as well as under this Regulation.

An application has been submitted by the companies Genencor International B.V. and Senson Oy for authorisation of the food enzyme β -amylase obtained from barley (*Hordeum vulgare*).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011⁴ 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 Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

⁴ 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 a safety assessment of the food enzyme β -amylase obtained from barley (*Hordeum vulgare*) in accordance with Article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

1.2. Information on existing authorisations and evaluations

According to the applicant, the authorities of Australia/New Zealand, Brazil, Canada, China, France, Mexico and the USA have evaluated and authorised the use of β -amylase obtained from barley, including malted barley, and/or other cereals in a number of food- and beverage-manufacturing processes. The conditions of use are not specified.

2. Data and methodologies

2.1. Data

The applicants have submitted a dossier in support of the application for authorisation of the food enzyme β -amylase obtained from barley (*Hordeum vulgare*).⁵ The food enzyme is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups.⁶

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, 2009a) and following the relevant existing guidelines from the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier for safety evaluation of a food enzyme' (EFSA, 2009b) 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 EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) 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 barley grain.

3.1.2. Chemical parameters

The molecular mass of 59.6 kDa reported in the dossier for the β -amylase from barley (*Hordeum vulgare*) in the food enzyme, was calculated from the amino acid sequence deduced from the cDNA sequence (UniProtKB – P16098⁷).

Data on chemical parameters of the food enzyme were provided for three commercial batches (Table 1). The average total organic solids (TOS) content of the three commercial batches was 49.3% (w/w); the values ranged from 47.4% to 51.9%. The TOS content is a calculated value of 100% food enzyme minus % water content minus % ash.

⁵ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2014-00731>

⁶ A 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.

⁷ Available from: <http://www.uniprot.org/uniprot/P16098>

Enzyme activity, expressed as degrees diastatic power ($^{\circ}$ DP), ranged from 1,828 to 2,033 $^{\circ}$ DP/g batch. The average enzyme activity/TOS ratio of the three commercial food enzyme batches was 3.95 $^{\circ}$ DP/mg TOS; the values ranged from 3.77 to 4.16 $^{\circ}$ DP/mg TOS. Considering the low variability of the enzyme activity/TOS ratio of the three batches, the average activity/mg TOS ratio of 3.95 $^{\circ}$ DP/mg TOS was used for subsequent calculations.

The protein pattern of the food enzyme was determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis. The protein profiles provided for three food enzyme batches were comparable. In addition to the band assigned to β -amylase by the applicant, i.e. slightly above 50 kDa, the presented gels showed two further major protein bands at \sim 38 and 12 kDa with similar intensities, and several other additional bands. The observed complexity of the protein profiles reflects the fact that the food enzyme is extracted from barley grain without further protein fractionation.

No analytical data on the activities of other enzymes in the food enzyme product were provided. Taking into account that the food enzyme is obtained from an edible part of a plant, the Panel considered this acceptable.

Table 1: Compositional data provided for three commercial batches of the food enzyme

| Parameter | Unit | Batches | | |
|----------------------------------|-----------------------|---------|-------|-------|
| | | 1 | 2 | 3 |
| β -Amylase activity | $^{\circ}$ DP/g batch | 1,970 | 1,828 | 2,033 |
| Protein | % | 17.9 | 16.6 | 18.6 |
| Ash | % | 2.4 | 2.3 | 2.3 |
| Water | % | 50.2 | 49.2 | 45.8 |
| TOS ^(a) | % | 47.4 | 48.5 | 51.9 |
| β -Amylase activity/mg TOS | $^{\circ}$ DP/mg TOS | 4.16 | 3.77 | 3.92 |

$^{\circ}$ DP: diastatic power degrees; TOS, total organic solids.

(a): TOS calculated as 100% – % water – % ash.

In addition to the data presented in Table 1, the applicant provided data on three other batches of the food enzyme, demonstrating that the contents of lead (< 0.05 mg/kg) and several mycotoxins (ochratoxin A, aflatoxins and *Fusarium* toxins) were below the respective limits of quantification.⁸

The applicant also provided analytical data on three other batches of the food enzyme, demonstrating that the gluten content was below the limit of quantification (██████████) of the applied enzyme-linked immunosorbent assay (ELISA)-based method.

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 present at not more than 30 colony forming units per gram.

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

3.1.3. Properties of the food enzyme

The food enzyme β -amylase catalyses the hydrolysis of 1,4- α -glycosidic linkages in polysaccharides and successively releases maltose units from the non-reducing ends of the chains. No cofactors are required.

The activity of β -amylase is quantified based on measurement of the reducing sugars released from soluble starch via hydrolysis and is expressed in degrees diastatic power ($^{\circ}$ DP/g). Quantitative measurement of the released reducing sugars is based on titration of Fehling's solution (copper II ions and tartrate ions) with a starch solution hydrolysed by the food enzyme (reaction conditions: pH = 4.6, temperature = 20 \pm 0.2°C, incubation time = 60 min) using methylene blue as an indicator.

The β -amylase was characterised regarding its activity dependence on temperature and pH. The pH profile was measured over a range of 3.5–8.0 at 20°C (25% relative activity at pH 3.5 and \sim 60% relative activity at pH 8.0, with an optimum pH between 4.7 and 5.5). The temperature profile was measured from 55°C to 80°C at pH 4.6. The temperature optimum was found to be between 55°C and

⁸ The limit of quantification for ochratoxin A is 0.2 μ g/kg food enzyme product, for all four aflatoxins (B1, B2, G1 and G2) is 0.1 μ g/kg food enzyme product, for two fusarium toxins (deoxynivalenol and nivalenol) is 20 μ g/kg food enzyme product, for three fusarium toxins (HT-2, T2 and zearalenone) is 10 μ g/kg food enzyme product.

63°C. The thermostability of β -amylase was measured under standard assay conditions after pre-incubation at pH 4.6 and different temperatures for up to 100 min. No enzyme activity could be measured after treatment for > 50 min at 67°C.

3.1.4. Information on the plant source material

Barley grain is routinely eaten in many parts of the world (for example, see Loftas et al., 1995). It is also commonly malted and then used as a key ingredient in alcoholic beverage production (e.g. beer and whisky). According to information provided by the applicant, barley grain used to extract the food enzyme fulfils the requirements of Regulation (EU) No 1881/2006⁹ setting maximum levels for certain contaminants in foods and of Regulation (EU) No 396/2005¹⁰ on maximum residue levels of pesticides in or on food and feed of plant and animal origin, and Regulation (EU) No 2015/1940¹¹ on maximum residue levels of ergot alkaloids in unprocessed cereals.

3.1.5. Manufacturing process

The manufacturing process comprises extraction and downstream processing. A comprehensive dataset 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 Regulation (EC) No 852/2004¹², with food safety procedures based on HACCP principles, and in accordance with current good manufacturing practice.

Prior to extraction, barley grains may be subjected to dehusking and/or malting. The food enzyme is obtained by extraction with water and separated from insoluble barley material by centrifugation and filtration.

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, [REDACTED] of TOS can be obtained from 1 kg of barley malt, which corresponds to a yield factor of [REDACTED].

The Panel considered the information provided on the raw materials and the manufacturing process as sufficient. The manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns.

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.

β -Amylase is specific in its action and is 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.

The data and the information provided indicate that β -amylase is inactivated and/or removed during the food manufacturing processes under the intended conditions of use, e.g. via thermal treatment or distillation.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups (Table 2).

⁹ Commission Regulation (EU) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.

¹⁰ Regulation (EC) No 396/2005 of the European Parliament and of the Council on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

¹¹ Commission Regulation (EU) 2015/1940 of 28 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia in certain unprocessed cereals and the provisions on monitoring and reporting. OJ L283, 29.10.2015, p. 4–6.

¹² Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 226, 25.6.2004, p. 3–21.

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

| Food manufacturing process ^(a) | Raw material | Recommended dosage of the food enzyme |
|--|--------------|---|
| Baking processes | Flour | 10–80 DP°/kg flour, corresponding to 2–20 mg TOS/kg flour |
| Brewing processes (beer) | Cereals | 200–1,000 DP°/kg malted barley or other cereals, corresponding to 50–250 mg TOS/kg malted barley or other cereals |
| Distilled alcohol production | Cereals | 200–1,000 DP°/kg cereals, corresponding to 50–250 mg TOS/kg cereals |
| Starch processing for the production of glucose syrups | Starch | 60–1,600 DP°/kg cereals, corresponding to 15–400 mg TOS/kg starch |

DP°: diastatic power degrees; TOS, total organic solids.

(a): 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.

In baking, the food enzyme is added to the raw materials during preparation of the dough. It is used to partially hydrolyse starch, resulting in a starch backbone with shorter chains, in non-branched glucan chains of lower molecular mass and in maltose. Its use provides maltose for yeast fermentation, improves the dough structure, ensures a uniform volume and better crumb structure, reduces viscosity and thus facilitates handling of the dough.

In brewing processes, the food enzyme is added during the mashing step. β -Amylase is used to convert liquefied starch into a maltose-rich solution, improving the amounts of fermentable sugars and thus increasing brewing yield.

In beverage alcohol (distilling) processes, the food enzyme is added during the slurry mixing step, in the liquefaction step and/or in the presaccharification step. β -Amylase is intended to be used to convert liquefied starch into a maltose-rich solution, to increase the amounts of fermentable sugars and the concentration of solids, which potentially results in higher alcohol yields.

In starch processes, β -amylase is added after the secondary liquefaction during the saccharification step, to convert liquefied starch into a maltose-rich solution, to ensure smooth and efficient processing, to facilitate separation and to ensure high-quality maltose syrup, resulting in energy savings in production and less waste water.

According to the applicant, the food enzyme is used at the minimum dosage necessary to achieve the desired reaction according to good manufacturing practice. In practice, the dosage applied by a food manufacturer will depend on the particular process (Table 2).

3.2. Dietary exposure

Following the EFSA Guidance Document on food enzymes (EFSA, 2009b), 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 barley comparable to the food enzyme–TOS, resulting from the consumption of barley-derived foods (herein referred to as source material TOS equivalent, 'SMT–Equivalent').

In both cases, food consumption data from the EFSA Comprehensive European Food Consumption Database (hereafter the EFSA Comprehensive Database¹³) were used.

Exposure estimates were calculated using the methodology described in the CEF Panel statement on the exposure assessment of food enzymes (EFSA CEF Panel, 2016). The assessment of the food processes covered in this opinion involved selection of relevant food groups and application of process and technical conversion factors (Appendix B), as appropriate (Section 3.2.3). These input data were subject to a stakeholder consultation through open calls,¹⁴ and adjusted in accordance with feedback received.

¹³ Available from: <http://www.efsa.europa.eu/en/food-consumption/comprehensive-database>

¹⁴ <http://www.efsa.europa.eu/en/data/call/161110>

3.2.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the Comprehensive Database has been populated with detailed national data on food consumption. Competent authorities in European countries provide EFSA with data regarding the level of food consumption by individual consumers, as taken from the most recent national dietary survey in their country (EFSA, 2011a).

The food consumption data gathered by EFSA were collected using different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used in exposure calculations, uncertainties might be introduced owing to possible subjects' underreporting and/or misreporting of consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Appendix A).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b).

3.2.2. Exposure assessment methodology

Chronic exposure was calculated based on individual consumption, averaged over the total survey period, excluding surveys with only one day per subject. 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, 2011a).

Exposure to the FE-TOS was calculated by multiplying values reported in Appendix B for each food category by their respective consumption amount per kilogram of body weight (kg bw) separately for each individual in the database.

The dietary exposure to the SMT-Equivalent was calculated by first estimating the intake of barley grain from all dietary sources (applying recipe and conversion fractions reported in Appendix C). Second, the so-derived intake of barley grain was converted into a barley fraction comparable to the food enzyme-TOS via application of a factor provided by the applicant to take into account the yield of the FE-TOS from the barley source (Section 3.1.5).

In both cases, the exposure per FoodEx category (Appendices B and C) was subsequently added to derive an individual total exposure per day. Finally, these exposure estimates were averaged over the number of survey days and normalised for individual body weight (bw), resulting in an individual average exposure/day per kg bw for the survey period. This was done for all individuals in the survey and per age group, resulting in distributions of individual average exposure per survey and population group. Based on these distributions, the mean and 95th percentile exposures were calculated per survey for the total population and per population group.

3.2.3. Exposure to food enzyme-TOS according to the intended use proposed by the applicant

Exposure to the FE-TOS was estimated based on intended uses and the recommended maximum use levels of the food enzyme-TOS provided by the applicant (Table 2). The FE-TOS exposure was calculated from foods produced involving baking and brewing processes.

Foods/ingredients derived through alcohol (distillation) processes and starch processing, i.e. spirits and glucose syrups, were excluded from the analysis. Experimental data on the significant removal (> 99%) of protein in the course of these processes have been provided (Documentation provided to EFSA, No.6). The Panel considered this evidence as sufficient to conclude that the presence of residual amounts of TOS after distilling or filtration and purification during processing is negligible.

Relevant food groups and/or individual foods were selected from the Comprehensive Database and were assumed to always contain the FE-TOS at the maximum recommended use level. This will result in an overestimation of exposure to FE-TOS.

To facilitate matching of the reported use levels for baking and brewing processes with foods identified in the Comprehensive Database, the selected foods were disaggregated to ingredient level as appropriate, and converted into the corresponding raw material, i.e. flour or grain, via the application of conversion factors (Appendix B). For example, consumption of 100 g of bread was converted into an intake of 70 g flour (recipe fraction of 0.7) and then multiplied by 20 mg TOS/kg flour, as provided by the applicant, to arrive at an exposure of 1.4 mg TOS/100 g bread.

Exposure to the FE-TOS was calculated by multiplying values reported for each food category by their respective consumption amount per kilogram of body weight separately for each individual in the database. Table 3 provides an overview of the derived exposure estimates. The average and 95th percentile exposure to the food enzyme-TOS per age class, country and survey are reported in Appendix E – Table 1. The contribution of the food enzyme-TOS from each FoodEx category to the total dietary exposure is indicated in Appendix E – Table 2.

Table 3: Summary of estimated dietary exposure to the food enzyme-TOS in six population groups

| Estimated exposure (mg/kg bw 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) | 0.0–0.06 (6) | 0.05–0.13 (10) | 0.06–0.12 (18) | 0.03–0.10 (17) | 0.06–0.36 (17) | 0.04–0.19 (14) |
| Min–max 95th percentile (number of surveys) | 0.07–0.17 (5) | 0.11–0.21 (7) | 0.10–0.22 (18) | 0.06–0.42 (17) | 0.19–1.48 (17) | 0.09–0.70 (14) |

bw: body weight.

3.2.4. Dietary exposure to SMT-Equivalent resulting from the consumption of barley-derived foods

The FoodEx categories likely to contain barley or be produced from barley were selected from the EFSA Comprehensive Database, at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b). The process of selecting relevant food groups was aided by obtaining qualitative information from the Mintel's Global New Products Database.¹⁵

Major FoodEx categories identified as containing barley or being derived from barley comprise beer, coffee imitates, breakfast cereals, barley-derived distilled spirits and malted beverages. Barley was found as a minor ingredient in some FoodEx categories, but due to a lack of detailed recipe information, these categories were not taken into account in the assessment (see Appendix D). Exclusion of these food categories results in a minor underestimation of intake. Appendix B provides an overview of the categories selected following this approach.

The selected FoodEx categories were converted from food as consumed (including ingredients in composite foods) to the SMT-Equivalent via application of the correction factors described in Appendix C. For example, consumption of a 100 g cereal bar was converted into an intake of 5 g of malted barley (5% of malted barley used as an ingredient), and the malted barley intake was further converted via application of the FAO conversion of 1.37, into an intake of raw barley grain of 6.85 g. Finally, the 6.85 g intake of raw barley grain was converted into 137 mg of SMT-Equivalent/100 g cereal bar by application of the yield factor described in Section 3.1.5.

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

Table 4: Summary of estimated dietary exposure to the SMT-Equivalent, resulting from the consumption of barley-derived foods, in six population groups

| Estimated intake (mg/kg bw 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) | 0.0–4.88 (6) | 0.08–14.72 (10) | 0.14–8.93 (18) | 0.38–7.09 (17) | 2.88–28.23 (17) | 1.17–13.85 (14) |
| Min–max 95th percentile (number of surveys) | 0.0–25.57 (5) | 0.0–35.16 (7) | 0.0–29.68 (18) | 1.92–40.90 (17) | 15.28–125.27 (17) | 8.98–58.04 (14) |

bw: body weight.

¹⁵ Available online: <http://www.mintel.com/global-new-products-database>

3.2.5. Comparison of the exposure estimates

Exposure to the food enzyme, calculated on the basis of food enzyme–TOS (Table 3), was similar to or lower than the exposure to the SMT–Equivalent (Table 4).

3.2.6. 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 5.

Table 5: Qualitative evaluation of the influence of uncertainties on the dietary exposure estimate

| Sources of uncertainties | Direction of impact | |
|---|---------------------|----------------------------|
| | Exposure to FE–TOS | Exposure to SMT–equivalent |
| <i>Model input data</i> | | |
| 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 | +/- | +/- |
| <i>Model assumptions and factors</i> | | |
| FoodEx categories included in the exposure assessment were assumed to always contain the food enzyme–TOS | + | NA |
| Exposure to food enzyme–TOS was always calculated based on the maximum recommended use level | + | NA |
| Selection of broad FoodEx categories for the exposure assessment | + | NA |
| Selection of FoodEx categories likely to contain barley for the intake assessment of a fraction of barley comparable to the food enzyme–TOS, based on the national food descriptors, recipe databases and Mintel Global New Products Database | NA | +/- |
| Use of FAO technical conversion factors: whenever a range is reported the median value was used | +/- | +/- |
| Range of yield factors provided by applicant, the mean value was used | NA | +/- |
| Yield factor of the food enzyme was available only for barley malt | NA | +/- |
| Use of recipe fractions in disaggregation FoodEx categories likely to contain barley and/or food likely to contain the food enzyme | +/- | +/- |

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

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 food enzyme–TOS, in particular, assumptions made on the occurrence and use levels of this specific food enzyme, is likely to have led to a considerable overestimation of the exposure. 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 exposure to the food enzyme–TOS is likely to be considerably lower than estimated, and consequently the margin between the estimate of exposure to the food enzyme–TOS and the SMT–Equivalent is likely to be higher than estimated.

3.3. Toxicological data

According to the Commission Implementing Regulation (EU) No 562/2012¹⁶, 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.

¹⁶ 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.

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, 2009b).

The Panel considers that these requirements are fulfilled, because:

- i) Barley grain is routinely eaten in many parts of the world and malted barley grain is a key ingredient in the production of alcoholic beverages.
- ii) Barley used for the production of the food enzyme complies with the legal requirements regarding limits of potential contaminants and residues. The manufacturing process of the food enzyme is not considered to introduce substances that could raise safety concerns.
- iii) The compositional data provided on the food enzyme are considered sufficient.
- iv) Exposure to the food enzyme, calculated on the basis of food enzyme–TOS, is similar to or lower than the exposure to the SMT–Equivalent.

3.4. Allergenicity

Barley is a source of allergens that can elicit occupational respiratory allergy (e.g. baker's asthma) (Sandiford et al., 1994), and a source of gluten that can elicit coeliac disease – a form of gluten intolerance.

3.4.1. β -Amylase and other proteins extracted from barley grain

Potential allergenicity of β -amylase from barley was assessed by comparing the amino acid sequence (Kreis et al., 1987) with those of known food 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 > 35% identity in a sliding window of 80 amino acids as the criterion, no matches were found.

With regard to the allergenicity of β -amylase from barley, conflicting data are presented in various allergen databases. In the AllergenOnline database,¹⁷ β -amylase from barley is not listed as an allergen, whereas in the WHO/IUIS Allergen Nomenclature database¹⁸ β -amylase from barley is indicated as Hor v 17¹⁹ and indicated as a food allergen with no further information. In the Allergome database,²⁰ β -amylase from barley is also listed as allergen Hor v 17, but according to the allergenicity scoring it is only found positive in non-functional testing like immunoglobulin (Ig)E/IgE immunoblot testing. Moreover, it is indicated that no data on oral-, bronchial-, nasal- and conjunctival-provocation testing and skin testing, as well as on functional testing, such as the basophil degranulation/activation test, are available.

β -Amylase from barley is described as an occupational respiratory allergen that can induce occupational asthma in bakers and millers (Sandiford et al., 1994). In the various allergen databases, several other barley proteins (e.g. Hor v 12, Hor v 15, Hor v 16 and Hor v 20) are also listed as being associated with mainly baker's asthma (Barber et al., 1989; Mena et al., 1992; Sanchez-Monge et al., 1992; Armentia et al., 1993). However, no allergic reactions to β -amylase and the other barley proteins upon oral exposure have been reported in the literature. Moreover, several studies have shown that adults suffering from occupational asthma can ingest respiratory allergens without acquiring clinical symptoms of food allergy (Brisman, 2002; Poulsen, 2004; Armentia et al., 2009).

3.4.2. Gluten proteins extracted from barley grain

In Regulation (EU) No 1169/2011²¹, barley is listed as gluten-containing cereal which therefore can elicit coeliac disease, a form of gluten intolerance. However, because gluten is poorly soluble in water,

¹⁷ Available from: www.allergenonline.org

¹⁸ Available from: www.allergen.org

¹⁹ Allergen nomenclature database: Hor v 17: Hor(deum) v(ulgare), the species name of barley and allocated index number.

²⁰ Available from: <http://allergome.org>

²¹ 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.

the food enzyme obtained via extraction of barley grain using an aqueous solution is expected not to contain gluten. The applicant provided analytical data on three batches of the food enzyme, demonstrating that the gluten content was below the limit of quantification (████████ food enzyme) of the applied ELISA-based method.

Based on the estimated dietary exposure to the food enzyme TOS in six population groups (Table 3), the highest potential exposure to gluten from the food enzyme was calculated (for adults) and amounts to ██████ per day, which is several orders of magnitude lower than the exposure from the gluten-free foods with the threshold value of 20 mg/kg set for the labelling of foods as 'gluten-free' specified in European Commission Regulation (EC) 41/2009²².

Several clinical studies performed in patients with coeliac disease indicated the safe thresholds for gluten content in 'gluten-free' products in a range of 20–100 mg/kg (Collin et al., 2004; Catassi et al., 2007). The threshold value of gluten in 'gluten-free' foods is set as 20 mg/kg in Commission Regulation (EC) No 41/2009¹³ for European population.

Overall, the Panel concluded that considering the manufacturing process and the intended conditions of use for this food enzyme, there is no concern with respect to allergenicity or food intolerance. Barley-based glucose syrups are exempted from labelling requirements according to Regulation (EU) 1169/2011.

4. Discussion

The food enzyme is obtained from barley grain, i.e. an edible plant source. The Panel considered the information provided on the source material, the manufacturing process and the composition of the food enzyme sufficient to conclude on its safety.

The food enzyme is intended to be used in several food-manufacturing processes: baking and brewing processes, distilled alcohol production, and starch processing for the production of glucose syrups. The use levels recommended for these food processes have been provided.

Exposure to the food enzyme–TOS was estimated using individual data from the EFSA Comprehensive Database. This exposure was similar to or lower than the exposure to a fraction of barley comparable to the food enzyme–TOS, resulting from the consumption of barley-derived foods.

In line with the requirements of the guidance document, the Panel accepted that there was no need for the provision of toxicological data for this food enzyme.

The gluten content of the food enzyme β -amylase derived from barley, which is listed as gluten-containing cereal in Regulation (EU) No 1169/2011, did not exceed the level of 20 mg/kg for gluten-free foods. In addition, the amino acid sequence of the food enzyme lacked similarity with those of known food allergens, and no food allergic reactions to the occupational respiratory allergen β -amylase were described in the literature. Taken together, the CEF Panel considers that under the intended conditions of use for this food enzyme, there is no concern with respect to allergenicity or food intolerance.

Conclusions

Based on the origin of the food enzyme from edible parts of barley, the enzyme-manufacturing process, the compositional and biochemical data provided, the allergenicity and dietary exposure assessment, the Panel considers that this food enzyme β -amylase obtained from barley by the companies Genencor International B.V. and Senson Oy does not raise safety concerns under the intended conditions of use.

Documentation provided to EFSA

- 1) Dossier 'Application for authorisation of β -amylase from barley (*Hordeum vulgare*)'. September 2014. Submitted by Genencor International B.V. and Senson Oy.
- 2) Summary report on technical data and dietary exposure related to β -amylase from the edible seed of barley (*Hordeum vulgare*). March 2015. Delivered by Hylobates Consulting (Rome, Italy) and BiCTm (Lodi, Italy).
- 3) Summary report on allergenicity related to β -amylase extracted from Barley (*Hordeum vulgare*). June 2015. Delivered by FoBiG GmbH (Freiburg, Germany).

²² Commission Regulation (EC) No 41/2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.

- 4) Additional information on raw material, thermos-stability and use level. July 2015. Provided by Genencor International B.V. and Senson Oy.
- 5) Additional information on the yield factor of the food enzyme. May 2016. Provided by Genencor International B.V. and Senson Oy.
- 6) Additional information on 'Food enzyme removal during the production of cereal based distilled alcoholic beverages' and 'Food enzyme carry-over in glucose syrups'. February 2017. Provided by the Association of Manufacturers and Formulators of Enzyme Products.

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Abbreviations

| | |
|----------------|--|
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CEF | EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids |
| °DP | diastatic power degree |
| EINECS | European Inventory of Existing Commercial Chemical Substances |
| ELISA | enzyme-linked immunosorbent assay |
| FAO | Food and Agricultural Organization of the United Nations |
| FE–TOS | food enzyme–TOS |
| HACCP | Hazard Analysis and Critical Control Points |
| IgE | immunoglobulin E |
| IUBMB | International Union of Biochemistry and Molecular Biology |
| SMT–Equivalent | source material TOS equivalent |
| TOS | total organic solids |
| WHO | World Health Organization |

Appendix A – 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, Finland, Germany, Italy, United Kingdom |
| Toddlers | From 12 months up to and including 35 months of age | Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, United Kingdom |
| Children ^(a) | From 36 months up to and including 9 years of age | Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, United Kingdom |
| Adolescents | From 10 years up to and including 17 years of age | Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, United Kingdom |
| Adults | From 18 years up to and including 64 years of age | Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, United Kingdom |
| The elderly ^(a) | From 65 years of age and older | Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, 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, 2011a).

Appendix B – FoodEx categories used to derive exposure estimates for the food enzyme–TOS and the respective conversion factors

| FoodEx code | FoodEx category | Conversion factor from FoodEx food group to raw material ^(a) | Recipe fraction ^(b) | mg TOS/kg flour |
|-----------------|--|---|--------------------------------|-----------------|
| A.01 | Grains and grain-based products (unspecified) | 0.8 | 1 | 20 |
| A.01.03 | Grain milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.001 | Wheat milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.001.001 | Wheat flour, brown | 1 | 1 | 20 |
| A.01.03.001.002 | Wheat flour, Durum | 1 | 1 | 20 |
| A.01.03.001.003 | Wheat flour, white | 1 | 1 | 20 |
| A.01.03.001.004 | Wheat flour, wholemeal | 1 | 1 | 20 |
| A.01.03.001.005 | Graham flour | 1 | 1 | 20 |
| A.01.03.001.006 | Wheat flour, gluten free | 1 | 1 | 20 |
| A.01.03.001.014 | Wheat starch | 1.2 | 1 | 20 |
| A.01.03.002 | Rye milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.002.001 | Rye flour, gluten free | 1 | 1 | 20 |
| A.01.03.002.002 | Rye flour, light | 1 | 1 | 20 |
| A.01.03.002.003 | Rye flour, medium | 1 | 1 | 20 |
| A.01.03.002.004 | Rye flour, wholemeal | 1 | 1 | 20 |
| A.01.03.003 | Buckwheat milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.003.001 | Buckwheat flour | 1 | 1 | 20 |
| A.01.03.004 | Corn milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.004.001 | Corn flour | 1 | 1 | 20 |
| A.01.03.004.003 | Corn starch | 1.3 | 1 | 20 |
| A.01.03.005 | Oat milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.005.002 | Oat flour | 1 | 1 | 20 |
| A.01.03.005.004 | Oat starch | 1.2 | 1 | 20 |
| A.01.03.006 | Rice milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.006.001 | Rice flour | 1 | 1 | 20 |
| A.01.03.006.002 | Rice flour white | 1 | 1 | 20 |
| A.01.03.006.003 | Rice flour, instant | 1 | 1 | 20 |
| A.01.03.006.004 | Rice starch | 1.2 | 1 | 20 |
| A.01.03.007 | Spelt milling products | 1 | 1 | 20 |
| A.01.03.008 | Other milling products (unspecified) | 1 | 1 | 20 |
| A.01.03.008.001 | Amaranth flour | 1 | 1 | 20 |
| A.01.03.008.002 | Barley flour | 1 | 1 | 20 |
| A.01.03.008.003 | Chapatti flour | 1 | 1 | 20 |
| A.01.03.008.004 | Flour mix, wheat/rye/barley/oats | 1 | 1 | 20 |
| A.01.03.008.005 | Millet flour | 1 | 1 | 20 |
| A.01.03.008.007 | Sorghum flour | 1 | 1 | 20 |
| A.01.04 | Bread and rolls (unspecified) | 1 | 0.7 | 20 |
| A.01.04.001 | Wheat bread and rolls | 1 | 0.7 | 20 |
| A.01.04.002 | Rye bread and rolls | 1 | 0.7 | 20 |
| A.01.04.003 | Mixed wheat and rye bread and rolls | 1 | 0.7 | 20 |
| A.01.04.004 | Multigrain bread and rolls | 1 | 0.7 | 20 |
| A.01.04.005 | Unleavened bread, crisp bread and rusk (unspecified) | 1 | 0.8 | 20 |
| A.01.04.005.001 | Crisp bread, rye wholemeal | 1 | 0.9 | 20 |

| FoodEx code | FoodEx category | Conversion factor from FoodEx food group to raw material ^(a) | Recipe fraction ^(b) | mg TOS/kg flour |
|-----------------|----------------------------------|---|--------------------------------|-----------------|
| A.01.04.005.002 | Crisp bread, rye, light | 1 | 0.9 | 20 |
| A.01.04.005.003 | Crisp bread, wheat, wholemeal | 1 | 0.9 | 20 |
| A.01.04.005.004 | Crisp bread, wheat, light | 1 | 0.9 | 20 |
| A.01.04.005.005 | Rusk, light | 1 | 0.9 | 20 |
| A.01.04.005.006 | Rusk, wholemeal | 1 | 0.9 | 20 |
| A.01.04.005.007 | Pita bread | 1 | 0.7 | 20 |
| A.01.04.005.008 | Matzo | 1 | 0.9 | 20 |
| A.01.04.005.009 | Tortilla | 1 | 0.7 | 20 |
| A.01.04.006 | Other bread | 1 | 0.7 | 20 |
| A.01.04.007 | Bread products | 1 | 0.7 | 20 |
| A.01.07 | Fine bakery wares (unspecified) | 1 | 0.5 | 20 |
| A.01.07.001 | Pastries and cakes (unspecified) | 1 | 0.5 | 20 |
| A.01.07.001.001 | Beignets | 1 | 0.15 | 20 |
| A.01.07.001.002 | Buns | 1 | 0.7 | 20 |
| A.01.07.001.003 | Cake from batter | 1 | 0.25 | 20 |
| A.01.07.001.004 | Cheese cream cake | 1 | 0.24 | 20 |
| A.01.07.001.005 | Cheese cream sponge cake | 1 | 0.24 | 20 |
| A.01.07.001.006 | Chocolate cake | 1 | 0.24 | 20 |
| A.01.07.001.007 | Chocolate cake with fruits | 1 | 0.24 | 20 |
| A.01.07.001.008 | Cream cake | 1 | 0.24 | 20 |
| A.01.07.001.009 | Cream cheese cake | 1 | 0.24 | 20 |
| A.01.07.001.010 | Cream custard cake | 1 | 0.24 | 20 |
| A.01.07.001.011 | Cream custard sponge cake | 1 | 0.24 | 20 |
| A.01.07.001.012 | Croissant | 1 | 0.5 | 20 |
| A.01.07.001.013 | Croissant, filled with chocolate | 1 | 0.5 | 20 |
| A.01.07.001.014 | Croissant, filled with cream | 1 | 0.5 | 20 |
| A.01.07.001.015 | Croissant, filled with jam | 1 | 0.5 | 20 |
| A.01.07.001.016 | Croquembouche | 1 | 0.15 | 20 |
| A.01.07.001.017 | Doughnuts | 1 | 0.24 | 20 |
| A.01.07.001.018 | Clair | 1 | 0.15 | 20 |
| A.01.07.001.019 | Flan | 1 | 0.5 | 20 |
| A.01.07.001.020 | Fruit cake | 1 | 0.6 | 20 |
| A.01.07.001.021 | Fruit pie | 1 | 0.15 | 20 |
| A.01.07.001.022 | Cheese pie | 1 | 0.15 | 20 |
| A.01.07.001.023 | Fruit tart | 1 | 0.15 | 20 |
| A.01.07.001.024 | Gingerbread | 1 | 0.6 | 20 |
| A.01.07.001.025 | Gougere | 1 | 0.15 | 20 |
| A.01.07.001.026 | Kringles | 1 | 0.25 | 20 |
| A.01.07.001.027 | Nut cream cake | 1 | 0.24 | 20 |
| A.01.07.001.028 | Pancakes | 1 | 0.25 | 20 |
| A.01.07.001.029 | Profiterole | 1 | 0.15 | 20 |
| A.01.07.001.030 | Pyramid cake | 1 | 0.25 | 20 |
| A.01.07.001.031 | Rhubarb flan | 1 | 0.15 | 20 |
| A.01.07.001.032 | Scone | 1 | 0.5 | 20 |
| A.01.07.001.033 | Sponge dough | 1 | 0.25 | 20 |
| A.01.07.001.034 | Sponge cake | 1 | 0.25 | 20 |
| A.01.07.001.035 | Sponge cake roll | 1 | 0.25 | 20 |

| FoodEx code | FoodEx category | Conversion factor from FoodEx food group to raw material ^(a) | Recipe fraction ^(b) | mg TOS/kg flour |
|-----------------|--|---|--------------------------------|-----------------|
| A.01.07.001.036 | Muffins | 1 | 0.25 | 20 |
| A.01.07.001.037 | Waffles | 1 | 0.25 | 20 |
| A.01.07.001.038 | Apple strudel | 1 | 0.15 | 20 |
| A.01.07.001.039 | Cream-cheese strudel | 1 | 0.24 | 20 |
| A.01.07.001.040 | Cheese pastry goods from puff pastry | 1 | 0.15 | 20 |
| A.01.07.001.041 | Croissant from puff pastry | 1 | 0.6 | 20 |
| A.01.07.001.042 | Brioche | 1 | 0.5 | 20 |
| A.01.07.001.044 | Lebkuchen | 1 | 0.6 | 20 |
| A.01.07.001.045 | Dumpling | 1 | 0.5 | 20 |
| A.01.07.001.046 | Cake marbled, with chocolate | 1 | 0.5 | 20 |
| A.01.07.001.047 | Marzipan pie | 1 | 0.25 | 20 |
| A.01.07.001.048 | Baklava | 1 | 0.15 | 20 |
| A.01.07.002 | Biscuits (cookies) | 1 | 0.9 | 20 |
| A.01.07.002.001 | Biscuits, sweet, plain | 1 | 0.9 | 20 |
| A.01.07.002.002 | Biscuits, chocolate filling | 1 | 0.81 | 20 |
| A.01.07.002.003 | Biscuits, cream filling | 1 | 0.81 | 20 |
| A.01.07.002.004 | Biscuits, fruit filling | 1 | 0.81 | 20 |
| A.01.07.002.005 | Biscuits, vanilla filling | 1 | 0.81 | 20 |
| A.01.07.002.006 | Butter biscuits | 1 | 0.81 | 20 |
| A.01.07.002.007 | Biscuit, iced | 1 | 0.81 | 20 |
| A.01.07.002.008 | Speculaas | 1 | 0.9 | 20 |
| A.01.07.002.009 | Biscuits, sweet, wheat wholemeal | 1 | 0.9 | 20 |
| A.01.07.002.010 | Biscuits, oat meal | 1 | 0.9 | 20 |
| A.01.07.002.011 | Biscuits, spelt meal | 1 | 0.9 | 20 |
| A.01.07.002.012 | Biscuits, salty | 1 | 0.9 | 20 |
| A.01.07.002.013 | Biscuits, salty, with cheese | 1 | 0.81 | 20 |
| A.01.07.002.014 | Sticks, salty | 1 | 0.81 | 20 |
| A.17.03.003 | Biscuits, rusks and cookies for children | 1 | 0.9 | 20 |
| A.18.04.001 | Find bakery products for diabetics | 1 | 0.5 | 20 |
| A.19.01.002 | Pizza and pizza-like pies | 1 | 0.3 | 20 |
| A14.01 | Beer and beer-like beverage | 1.37 | 0.19 | 250 |
| A.14.01.001 | Beer, strong | 1.37 | 0.265 | 250 |
| A.14.01.002 | Beer, regular | 1.37 | 0.19 | 250 |
| A.14.01.003 | Beer, light (reduced alcohol content) | 1.37 | 0.135 | 250 |
| A.14.01.004 | Beer, alcohol-free | 1.37 | 0.135 | 250 |
| A.14.01.005 | Beer-like beverages (malt drink) | 1.37 | 0.19 | 250 |

TOS: total organic solids.

(a): Available at see <http://www.fao.org/fileadmin/templates/ess/documents/methodology/tcf.pdf>

(b): Derived from publically available recipe information, and/or food label information (such as the Mintel's Global New Products Database <http://www.mintel.com/global-new-products-database>).

Appendix C – FoodEx categories used to derive intake estimates for the raw barley grain and the respective conversion factors

| FoodEx code | FoodEx name | FAO conversion factor from FoodEx food group to raw barley grain ^(a) | Proportion of barley used as ingredient or in production of food ^(b) (%) |
|-----------------|---------------------------------------|---|---|
| A.01.02.002 | Barley grain | 1 | 100 |
| A.01.02.002.001 | Barley grain, whole | 1 | 100 |
| A.01.02.002.002 | Barley, pearled | 2.525 | 100 |
| A.01.03.008.002 | Barley flour | 3.09 | 100 |
| A.01.03.008.004 | Flour mix, wheat/rye/barley/oats | 3.09 | 25 |
| A.01.04.006 | Other bread ^(c) | 3.09 | 35 |
| A.01.04.006.004 | Muesli bread | 3.09 | 0.2 |
| A.01.05.008 | Pasta, mixed cereal flour | 3.09 | 15 |
| A.01.06.001.001 | Barley flakes | 1.39 | 100 |
| A.01.06.001.008 | Mixed cereal flakes | 1.39 | 30 |
| A.01.06.002 | Muesli | 1 | 9 |
| A.01.06.003 | Cereal bars | 1.37 | 5 |
| A.01.06.006.001 | Barley grits | 2.525 | 100 |
| A.02.11.003 | Cocoa beverage-preparation, powder | 1.7 | 73 |
| A.01.06.007.005 | Barley porridge | 2.525 | 7.4 |
| A.02.13.005 | Barley coffee | 1 | 100 |
| A.02.13.008 | Mixture of coffee imitates | 1 | 30 |
| A.06.09.003.001 | Blood sausage | 1 | 5 |
| A.13.04 | Coffee imitates beverage | 1 | 5 |
| A.13.05.001 | Hot chocolate | 1.37 | 8 |
| A.14.01 | Beer and beer-like beverage | 1.37 | 19 |
| A.14.01.001 | Beer, strong | | 26.5 |
| A.14.01.002 | Beer, regular | | 19 |
| A.14.01.003 | Beer, light (reduced alcohol content) | | 13.5 |
| A.14.01.004 | Beer, alcohol-free | | 13.5 |
| A.14.01.005 | Beer-like beverages (malt drink) | | 19 |
| A.16.04.014 | Malt extract | 1.72 | 100 |
| A.19.10.002 | Grain soup | 1 | 3.2 |

(a): Available at see <http://www.fao.org/fileadmin/templates/ess/documents/methodology/tcf.pdf>

(b): Derived from publically available recipe information, and/or food label information (such as the Mintel's Global New Products Database <http://www.mintel.com/global-new-products-database>).

(c): Only if barley is clearly indicated in the original food name were surveys included in the food category 'other bread'.

Appendix D – Food groups excluded from the intake assessment of barley grain

| FoodEx code | FoodEx name | Reasons for exclusion ^(a) |
|--|--|--|
| One reason common to all food groups listed in this table is that the barley component cannot be identified in the FoodEx nomenclature at the same or higher hierarchy level | | |
| A.01.04.005 | Unleavened bread, crispbread and rusk | 'Melba toast, malted barley' from Italy, 'crispbread wheat or rye barley fruit' from Sweden Further checking against the descriptor could not identify barley |
| A.06.09.004 | Cooked smoked sausage | 'Sausage stuffed with pearl barley' from Finland A single item under this FoodEx might be relevant, however, many others certainly do not contain barley |
| A08.02.001.002 | Chocolate and fruit-flavoured milk | Neither the food descriptor nor the food labels can identify barley or barley malt |
| A.10.04.001 | Candies, with sugar | 'Boiled sweets barley sugar butterscotch glacier mints hard c' from UK, Barley is a natural flavouring on food labels |
| A.12.02.005 | Juice concentrate, currants (black) | 'Conc barley water low cal not blackcurrant' from UK It contains barley malt extract, the amount is not known, but is assumed to be negligible, based on recipe information |
| A.13 | Non-alcoholic beverage | 'Barley water, concentrated' from Ireland It contains barley malt extract, the amount is not known, but is assumed to be negligible, based on recipe information |
| A.17.05 | Cereal-based food for infants and young children | Barley component cannot be identified in the ingredient list of several corresponding foods |
| A.17.06.001 | Ready-to-eat meal for children, vegetable based | |
| A.17.06.002 | Ready-to-eat meal for children, cereal based | |
| A.17.06.005 | Fruit pure, for children | |
| A.18.03.001 | Carbohydrate-rich energy food products for sports people | Containing small amount of malt extract-based on food labels |
| A.19.01.003.006 | Pasta, cooked, meat and vegetable filling | 'Barley-shaped pasta oven with lamb meat in tomato sauce and butter' from Greece The original language showed that it contains soya not barley |
| A.19.05 | Meat-based meals | 'Hash made with EG barley offal' from Sweden A single item under this FoodEx might be relevant, however, many others certainly do not contain barley |
| A.14.06.002 | Whisky | The amount of dry matter is negligible |
| A.19.10.001 | Vegetable/herb soup | 'Stock bouillon with pot barley' or 'vegetable soup with pot barley' from Latvia Barley is a natural flavouring on food labels |

(a): Based on publicly available recipe information, and/or food label information (such as the Mintel's Global New Products Database <http://www.mintel.com/global-new-products-database>).
'Food descriptor' refers to the names of foods used by national dietary surveys included in the EFSA Comprehensive Database.

Appendix E – 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 <http://onlinelibrary.wiley.com/wol1/doi/10.2903/j.efsa.2017.4756/supinfo>).

The file contains four sheets, corresponding to four tables.

Table 1: Average and 95th percentile exposure to the food enzyme–TOS per age class, country and survey.

Table 2: Contribution of food categories to the dietary exposure to the food enzyme–TOS per age class, country and survey.

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

Table 4: Contribution of food categories to the dietary exposure of the SMT–Equivalent per age class, country and survey.