Safety evaluation of a β-amylase food enzyme obtained from wheat (Triticum spp.)

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Abstract

The food enzyme considered in this opinion is a β-amylase (EC 3.2.1.2), obtained from the grain of wheat (Triticum spp.) by Roquette (France). The β-amylase is intended to be used in starch processing for production of glucose syrups containing maltose to be used as a food ingredient. Since the presence of residual amounts of total organic solids (TOS) in glucose syrups after filtration and purification during starch processing is negligible, no dietary exposure was calculated. As the food enzyme is derived from edible parts of wheat, no toxicological tests are required. Wheat is known as a gluten-containing cereal. However, the gluten content of the food enzyme was shown to be below the limit of quantification 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 the β-amylase and the sequences of known food allergens; no match was found. Although β-amylase from wheat is described as a potential occupational respiratory allergen, and oral wheat challenges in wheat allergic patients may result in clinical symptoms, the enzyme and the low levels of other wheat proteins will be removed from the final food ingredients through a downstream purification process. Based on the origin of the food enzyme from edible parts of grain, the manufacturing process, and the compositional and biochemical data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

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Keywords: food enzyme, β-amylase, EC 3.2.1.2, Triticum spp., wheat

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

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1. Introduction

The Article 3 of the Regulation (EC) No 1332/2008 provides definitions for ‘food enzyme’ and ‘food enzyme preparation’.

‘Food enzyme’ means a product obtained from plants, animals or microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

‘Food enzyme preparation’ means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes entered 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 established 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:

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;

iii) 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 an approval via a Union 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 Union list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes. According to Regulation (EC) No 1332/2008 on food enzymes, a food enzyme which falls within the scope of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed should be authorised in accordance with that Regulation as well as under this Regulation.

An application has been submitted by the company Roquette for the authorisation of the food enzyme β-amylase obtained from wheat (Triticum spp.).

Following the requirements of Article 12.1 of Commission Regulation (EU) No 234/2011 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.

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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 the safety assessment on the food enzyme \( \beta \)-amylase obtained from wheat (\textit{Triticum} spp.) 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 France have evaluated and authorised the use of \( \beta \)-amylase obtained from wheat in the production of glucose syrups in the starch processing industry and the alcohol production and brewing industry.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of its application for the authorisation of the food enzyme \( \beta \)-amylase from wheat (\textit{Triticum} spp.). The food enzyme is intended to be used in starch processing.

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 Guidances 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 by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) for the evaluation of the application for authorisation of the food enzyme \( \beta \)-amylase.

3. Assessment

3.1. Technical data

3.1.1. Identity of the food enzyme

- IUBMB nomenclature: \( \beta \)-amylase
- Systematic name: 4-\( \alpha \)-D-Glucan maltohydrolase
- Synonyms: Saccharogen amylase, glycogenase, 1,4-\( \alpha \)-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 edible grain of wheat.

3.1.2. Chemical parameters

The molecular mass of approximately 55 kDa reported for \( \beta \)-amylase from wheat species \textit{Triticum urartu}, was calculated from the amino acid sequence (M8B5G5 UniProtKB\(^5\)), and was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analyses.

Data on the chemical parameters have been provided for three commercial food enzyme preparation batches (Table 1).

The total organic solids (TOS) content is a calculated value derived as 100% minus % water minus % ash minus % diluents. The average TOS content of three commercial food enzyme preparation batches was 16.3% (w/w); the values ranged from 16% to 17% (Table 1). The batches presented in Table 1 were concentrates stabilised by the addition of sodium carbonate and glycerol and preserved by addition of potassium sorbate.

The average enzyme activity/TOS ratio of the food enzyme, expressed as degrees diastatic power (\(^\circ\)DP) (see Section 3.1.3), was 7.9 \(^\circ\)DP/mg TOS.

\(^5\) http://www.uniprot.org/uniprot/M8B5G5
The protein pattern of the food enzyme was determined using SDS-PAGE analysis. The protein profiles provided for three food enzyme batches were comparable. Apart from the band assigned to the β-amylase, i.e. 53 kDa, the presented gels showed another major protein band at approximately 10 kDa and several other bands. The observed complexity of the protein profiles reflects the fact that the food enzyme is extracted from wheat grain without further protein fractionation.

Data on α-amylase activity, being a side activity of the food enzyme, have been provided. The average α-amylase activity was 3,926 α-amylase Units/L; the values ranged from 3,238 to 5,148 α-amylase Units/L.

No analytical data on the side 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.

The applicant provided data on seven batches of the food enzyme preparation demonstrating that the contents of lead and other heavy metals (As, Cd and Hg) were below the specification levels set for food additives (Pb: 10 mg/kg; As: 3 mg/kg; Cd and Hg: 1 mg/kg) (Regulation (EU) No 231/2012).6

The applicant also provided analytical data on five batches of the food enzyme preparation, demonstrating that the gluten content was below the limit of quantification (5 mg/kg) of the applied enzyme-linked immunosorbent assay (ELISA)-based method for all five batches.

Furthermore, the applicant provided analytical data on the concentrations of histamine (a biogenic amine naturally occurring in wheat grain) for three batches, which was 40–50 mg/kg and for sulphite 200–300 mg/kg.

Data were also provided for potential contaminants (e.g. ochratoxin A, deoxynivalenol (DON), zearalenone) as well as for pesticides’ residues in these three batches demonstrating that they comply with the legal requirements implied by the Regulation (EC) No 1881/20067 and the Regulation (EC) No 396/20058, respectively.

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

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

### 3.1.3. Properties of the food enzyme

β-amylase catalyses the hydrolysis of 1,4-α-glycosidic linkages in amylose and amylopectin and releases successively maltose units from the non-reducing ends of the chains. The β-amylase from the edible grain of wheat does not require cofactors.

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6 Regulation (EU) No 231/2012 of the European Parliament and of the Council of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83/1, 22.3.2012.

7 Commission Regulation (EC) No 1881/2006 setting maximum levels of certain contaminants in foodstuffs.

8 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.
The β-amylase activity is quantified based on the measurement of reducing sugars released from hydrolysis of soluble starch and is expressed as °DP/mL. One unit of activity is defined as the amount of enzyme contained in 0.1 mL of a solution at 5% of the enzyme preparation that produces sufficient reducing sugars to reduce 5 mL of Fehling's solution when the sample is incubated with 100 mL of starch substrate at 20°C for 1 h.

The α-amylase activity is quantified based on the hydrolysis of starch and is expressed in α-amylase Units/L. The activity is measured relative to a standard. One unit of amylase activity is defined as the amount of enzyme catalysing the hydrolysis of 1 μmol glucosidic linkage per minute under the conditions of the assay.

The β-amylase has been characterised regarding its activity depending on temperature and pH. The temperature profile has been measured from 20 to 70°C. The β-amylase is active at temperatures up to 70°C (with an optimum of 50-60°C at pH 5). The pH profile has been measured within a pH range of 3.0-8.0 (with an optimum of 3.5 at 20°C). The thermostability of the β-amylase was tested over the range of 25-70°C after incubation at different temperatures at pH 5 up to 60 min. The activity itself was measured under standard conditions. The food enzyme is stable at 25°C while it shows a decrease of 15% in activity after 30 min incubation at 45°C. Complete inactivation of the β-amylase activity is attained after 4 min incubation at 70°C.

3.1.4. Information on the plant source material

Wheat (Triticum spp.) is routinely consumed as a staple plant food in many regions of the world (FAO, 1995). The food enzyme is obtained from grains of wheat purchased from producers from the EU. A quality surveillance plan for the wheat source material has been established to ensure that only wheat grains, fit for human consumption, are obtained for use in the manufacture of wheat β-amylase food enzyme. The surveillance plan consists of analysing the raw material for potential contaminants and impurities, ensuring compliance with the relevant EU legislations and contaminants limits. Potential contaminants that may stem from the wheat source material include mycotoxins, heavy metals and pesticide residues as indicated in Section 3.1.2.

3.1.5. Manufacturing process

The manufacturing process comprises an extraction process and downstream processing. A comprehensive data set related to the manufacturing process including a list of raw materials used and a flow diagram was provided. The food enzyme is manufactured in accordance with Regulation (EC) No 852/20049, with food safety procedures based on Hazard Analysis and Critical Control Points (HACCP) principles, and in accordance with current Good Manufacturing Practice (GMP).

3.1.5.1. Information relating to the milling and extraction process

Prior to extraction, wheat grains are milled to remove the wheat bran and to produce wheat flour. Water is then added to the wheat flour to agglomerate gluten. Additional water is added and the gluten is removed through sieving, resulting in a starch slurry. Fibres and gums are then removed from the starch slurry through sieving. The starch is removed from the resulting mixture through two rounds of centrifugation, leaving a wheat starch effluent containing the soluble fractions of wheat, including β-amylase.

The mixture is then subjected to tangential membrane microfiltration to remove the insoluble fractions and colloids, and any potential contaminating microorganisms from the soluble fractions of wheat, in order to obtain a clear solution containing β-amylase.

9 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.
An ultrafiltration process is applied to concentrate the \( \beta \)-amylase enzyme and to remove residual salts, sugars and other impurities from the concentrate. The product is then stored in a standardisation tank. Potassium sorbate is added as a preservative, and glycerol and sodium carbonate are added for enzyme stabilisation purposes to produce the final wheat \( \beta \)-amylase enzyme preparation. The formulation ingredients comply with Regulation (EU) 231/2012.

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

3.1.6. Reaction and fate in food

The \( \beta \)-amylase catalyses the successive cleavage of 1,4-\( \alpha \)-glycosidic linkages in the starch polysaccharides amylose and amylpectin, releasing maltose units from the non-reducing ends of the starch chains.

\( \beta \)-amylase is specific in its action, not known to catalyse other reactions than the hydrolysis of starch components, amylpectin and amylose, into maltose and limit dextrins. These reaction products are naturally present in starch-containing foods.

Experimental evidence was provided that the \( \beta \)-amylase is removed from glucose syrups (below the limit of quantification of the applied immunoassay) through the purification steps involving carbon adsorption and the use of an ion exchange resin.

3.1.7. Case of need and intended conditions of use

The food enzyme is intended to be used in starch processing for the production of glucose syrups containing maltose, which are further used as a food ingredient. The intended uses provided by the applicant are listed in Table 2.

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

| Starch processes (glucose syrups with high maltose content) | Up to 44,000 °DP/kg liquefied starch, corresponding to 5,570 mg TOS/kg liquefied starch |
|-----------------------------------------------------------|----------------------------------------------------------------------------------|
| Starch processes (glucose syrups containing maltose)       | Up to 26,000 °DP/kg liquefied starch, corresponding to 3,291 mg TOS/kg liquefied starch |
| Starch processes (dried glucose syrups containing maltose) | Up to 6,000 °DP/kg liquefied starch, corresponding to 759 mg TOS/kg liquefied starch |

TOS: total organic solids.

In starch processing, the food enzyme is added to liquefied starch following an initial hydrolysis step (liquefaction), in order to convert liquefied starch into glucose syrups that contain maltose.

As stated in the dossier, the food enzyme is used at the minimum dosage necessary to achieve the desired reaction according to GMP. The dosage applied in practice by a food manufacturer depends on the particular process.

3.2. Dietary exposure

For this application, the intended use of the wheat-derived \( \beta \)-amylase is to hydrolyse starch in order to produce glucose syrups containing maltose. Experimental data on the significant removal (\( > 99\% \)) of protein in the course of this process have been provided (AMFEP, 2017). Experimental evidence shows that the \( \beta \)-amylase is removed from glucose syrups was also provided (Section 3.1.6). The Panel considered this evidence as a sufficient to conclude that the presence of residual amounts of TOS after filtration and purification during processing is negligible. Consequently, no exposure was calculated.
3.3. Toxicological data

According to the Commission Implementing Regulation (EU) No 562/2012, 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 to be 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, 2009b).

The Panel considers these requirements being fulfilled, because:

i) the food enzyme is derived from edible parts of wheat (i.e. grain),
ii) the information on chemical and microbiological specification of different batches of the food enzyme, as well as its extraction process was sufficient to conclude on its safety,
iii) wheat grain has a well-known history of consumption,
iv) since the presence of residual amounts of TOS in sugar syrups after filtration and purification during starch processing is negligible, no quantitative dietary exposure was made.

3.4. Allergenicity

Wheat is a source of allergens that can elicit allergies, both occupational respiratory allergy (Baker’s asthma) and food allergy (Tatham and Shewry, 2008; Scibilia et al., 2006; Sotkovski et al., 2011), 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 wheat

Potential allergenicity of β-amylase from wheat was assessed by comparing the amino acid sequence (Kreis et al., 1987) with the sequences 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 higher than 35% identity in a window of 80 amino acids as the criterion; no matches were found.

β-amylase from wheat is described as a potential occupational respiratory allergen based on the recognition by immunoglobulin E (IgE) antibodies from wheat allergic patients suffering from Baker’s asthma (Sandiford et al., 1994; Sotkovsky et al., 2008). However, no allergic reactions to β-amylase from wheat upon oral exposure have been reported in literature.

Wheat food allergy in children and infants has been confirmed by a number of Double-Blind Placebo-Controlled Food-Challenges (DBPCFC) with wheat (e.g. Ellman et al., 2002). In addition, Scibilia et al. (2006) have shown that DBPC-wheat challenges performed with 27 suspected adult wheat allergic patients, also resulted in clinical symptoms of food allergy in 13 out of the 27 adult patients, of which seven showed clinical symptoms to less than 1.6 g wheat.

However, while the food enzyme may be potentially allergenic in susceptible individuals, the β-amylase and the low levels of other wheat proteins will be removed from the final food products through a downstream purification process and not present at detectable levels in the final products (< limit of quantification of 1 mg/kg). Wheat-based glucose syrups are exempted from the labelling requirements according to the Regulation (EU) 1169/2011.

3.4.2. Gluten proteins extracted from wheat

In the Regulation (EU) No 1169/2011, wheat is listed as gluten-containing cereal. However, since gluten is poorly soluble in water, the food enzyme obtained via extraction of wheat grain using an aqueous solution, is expected not to contain gluten. The applicant provided analytical data on five batches of the food enzyme demonstrating that the gluten content was below the limit of...
quantification (5 mg/kg food enzyme) of the applied immunoassay. 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 41/2009\textsuperscript{12} for European population.

Overall, the Panel concluded that considering the manufacturing process and the intended conditions of use, there is no concern with respect to food intolerance or allergenicity for this food enzyme.

4. Discussion

The food enzyme is obtained from wheat 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 starch processing for the production of glucose syrups containing maltose, which are further applied as a food ingredient. The use levels recommended for this food processing have been provided.

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.

Although wheat is known as a gluten-containing cereal (Regulation (EU) No 1169/2011), the gluten content of this enzyme did not exceed the level of 20 mg/kg for gluten-free food. Furthermore, the allergenicity of \(\beta\)-amylase was evaluated by searching for similarity of the amino acid sequence to those of known food allergens and no match was found. The Panel noted that \(\beta\)-amylase from wheat is described as a potential occupational respiratory allergen, and oral wheat challenges in wheat allergy patients may result in clinical symptoms. However, the enzyme and the low levels of other wheat proteins are removed from the final food products through a downstream purification process. Wheat-based glucose syrups are exempted from the labelling requirements according to the Regulation (EU) 1169/2011.

Considering the scientific opinion on risk-based control of biogenic amine formation in fermented foods of the Panel on Biological Hazards (EFSA BIOHAZ Panel, 2011) and taking into account the exclusive use of the enzyme in the starch processing and the assumption that this process will result in the negligible amount of food enzyme TOS in the final product, the Panel considered the amount of histamine in the food enzyme as not being of concern.

The Regulation (EU) No 1130/2011 gives a maximum permitted level (MPL) of 2,000 mg/kg sulfite for enzyme preparations. The determined levels of 200–300 mg/kg of sulfite are well below this MPL.

Overall, the Panel concluded that considering the manufacturing process and the intended conditions of use, there is no concern with respect to food intolerance or allergenicity for this food enzyme.

Conclusions

Based on the described manufacturing process and the compositional and biochemical data provided, and taking into account the negligible intake, the Panel concluded that this \(\beta\)-amylase food enzyme obtained from wheat by Roquette does not raise safety concern under the intended conditions of use.

Documentation provided to EFSA

1) Dossier ‘Application for authorisation of \(\beta\)-amylase (\textit{Triticum} spp.).’ March 2015. Submitted by Roquette.

2) Summary report on technical data and dietary exposure for \(\beta\)-amylase derived from the edible grain of wheat (\textit{Triticum} spp.). Delivered by Hylobates Consulting (Rome, Italy) and BiCT (Lodi, Italy).

3) AMFEP (Association of Manufacturers and Formulators of Enzyme Products), 2017. Food enzyme carry-over in glucose syrups. 22 February 2017. Unpublished document.

\textsuperscript{12} Commission Regulation (EC) No 41/2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.
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Abbreviations

CAS Chemical Abstracts Service
CEF EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CFU colony Forming Units
DBPCFC Double-Blind Placebo-Controlled Food-Challenges
DON deoxynivalenol
DP diastatic power
EC Enzyme Commission
EINECS European Inventory of Existing Commercial Chemical Substances
ELISA enzyme-linked immunosorbent assay
FAO Food and Agricultural Organization
GMO genetically modified organism
GMP Good Manufacturing Practice

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| Acronym | Definition                                      |
|---------|-----------------------------------------------|
| HACCP   | Hazard Analysis and Critical Control Points   |
| IgE     | immunoglobulin E                              |
| IUBMB   | International Union of Biochemistry and Molecular Biology |
| JECFA   | Joint FAO/WHO Expert Committee on Food Additives |
| MPL     | maximum permitted level                        |
| OECD    | Organisation for Economic Cooperation and Development |
| SDS-PAGE| sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| TOS     | total organic solids                           |
| WHO     | World Health Organization                      |