



## Food additive submission checklist – Food enzyme

### Instructions

This checklist is divided into several main sections, each with a specific set of requirements:

- Section 1: Administrative
- Section 2: Chemical safety
- Section 3: Assessment of potential dietary exposure for the general population
- Section 4: Toxicological safety
- Section 5: Allergenic safety
- Section 6: Nutritional safety
- Section 7: Molecular biological and microbiological safety
- Section 8: Additional considerations
- Section 9: Proposed food additive label

As you work through each section, check off each completed item. If you have any questions concerning the items in this checklist, please contact the Submission Management and Information Unit (SMIU) via the following email address: [smiu-ugdi@hc-sc.gc.ca](mailto:smiu-ugdi@hc-sc.gc.ca). For information on how to prepare a food additive submission, please refer to Health Canada's guidance document, titled [A Guide for the Preparation of Submissions on Food Additives](#). If further guidance is required, it is strongly recommended to request a [pre-submission consultation](#) with the Food Directorate.

### How to submit

The food additive submission should be sent electronically through the [Online Application Form for Pre-Market Submissions to the Food Directorate](#). Please review the guidance document, titled [How to Complete the Online Application and Transport Form for Pre-Market Submissions to the Food Directorate](#).

**Note:** Your submission must be organized following the order and titles of the main sections (Sections 1 to 9) set out above. Failure to do so will result in the closure of your submission.

If you cannot provide each piece of information required by the checklist (i.e., if you do not check off for one of the boxes in the checklist), you must provide a written explanation to justify why each piece of missing information is not provided to support the safety assessment of the food enzyme. Your explanation must be provided in the corresponding section of your submission, not in the checklist.

The submission should not omit, without explanation, any reports of investigations or planned investigations that could be used in an evaluation of the safety of the food additive.

All fields in this document are required\*.

Section 1: Administrative	
1.1 Name of petitioner (manufacturer, company, consultant, importer, etc.)	
1.2 Name of food enzyme	
1.3 Cover letter	
This cover letter must include the following information:	
	Title of submission <sup>1</sup>
	Date cover letter is signed
	Submission type (i.e., food additive)
	Submission sub-type (i.e., food enzyme)
	Description of sub-type – please describe the request (e.g., request to permit a new source of an already-permitted food enzyme; request to permit an already-permitted food enzyme and source in new foods)
	Food enzyme name, as intended to be listed in the <a href="#">list of permitted food enzymes</a>
	Food enzyme source, as intended to be listed in the <a href="#">list of permitted food enzymes</a>
	Executive summary explaining the technological purpose or function of the food enzyme, types of foods in which it is proposed for use, and the maximum recommended levels of use in each of those foods
	Reference to related submission number (e.g., pre-submission, resubmission), where applicable
	Signature
1.4 Authorization forms	
<b>Note:</b> A signed DPA form need only be provided if the primary contact is a designated party (e.g., consultant) authorized to act on behalf of the petitioner. The primary contact serves as the individual to which all correspondence from the Food Directorate will be sent.	
	Signed <a href="#">Designated Party Authorization (DPA) Form</a>

<sup>1</sup> Please follow the naming convention for the Title: [Food enzyme name] from [source] as a food enzyme in [food category/categories], e.g., Cellulase from *Aspergillus niger* var. as a food enzyme in distillers' mash, liquid coffee concentrate, natural flavour and colour extractives, and spice extracts; Glucoamylase from *Rhizopus oryzae* var. in various foods

<b>Section 2: Chemical safety</b>	
<b>2.1 Description of the food enzyme</b>	
<b>2.1.1 Identity of the food enzyme</b>	
	(a) Common or non-proprietary name
	(b) Nomenclature and classification according to the IUBMB (International Union of Biochemistry and Molecular Biology)
	(c) CAS (Chemical Abstracts Service) registry number, where applicable
	(d) INS (International Number System) number, where applicable
	(e) EC (Enzyme Commission) number
	(f) Molecular weight <sup>2</sup>
	(g) Amino acid sequence <sup>3</sup>
	(h) TOS (Total Organic Solids) content of the recovered food enzyme concentrate
<b>2.1.2 Details of the commercial food enzyme preparation</b>	
	(a) Trade name
	(b) Full, quantitative chemical composition
	(c) Description of the technological purpose of each ingredient
	(d) Confirmation that each ingredient is of food-grade quality
	(e) TOS content
<b>2.1.3 Method of manufacture</b>	
	(a) Detailed explanation of each step <sup>4</sup>
	(b) Identification of all raw materials used
	(c) Description of the technological purpose of each raw material used
	(d) Identification of the step in the manufacturing process at which each raw material is used
	(e) Confirmation and supporting rationale that all raw materials are of food-grade quality

<sup>2</sup> Provide the molecular weight of (1) the transcribed protein based on translation of its gene sequence and, where applicable, (2) the functional enzyme following any and all post-translational modifications.

<sup>3</sup> Provide the amino acid sequence of (1) the transcribed protein based on translation of its gene sequence and, where applicable, (2) the functional enzyme following any and all post-translational modifications.

<sup>4</sup> Please ensure to highlight and explain any steps that represent a barrier for microbial growth.

	(f) Residual level of materials used during manufacture (e.g., antifoaming agents, flocculating agents, antimicrobials) in the food enzyme concentrate. If no residues are expected in the food enzyme concentrate, a detailed scientific rationale (chemistry-based) explaining the absence of residues must be provided.
2.1.4 Chemical and physical properties	
	(a) Description of the chemical reaction catalyzed by the food enzyme, ensuring to identify the substrate(s) on which the food enzyme acts and the reaction product(s) formed as a result of the reaction
	(b) Data on the stability of the food enzyme under different pH and temperature conditions over time <sup>5</sup>
	(c) Comments and supporting rationale on the fate (e.g., active, denatured, <sup>6</sup> decreased in level <sup>7</sup> ) of the food enzyme in the food that is manufactured with the food enzyme
	(d) Identification and level of any substance in the food (e.g., degradation products) that results from the use of the food enzyme
	(e) Identification of the average activity of the food enzyme
	(f) Definition of the units in which food enzyme activity is reported
	(g) Explanation of how food enzyme activity is measured
	(h) Identification of any side enzymatic activities of significance produced by the source organism
2.1.5 Specifications	
	(a) Specifications (chemical and microbiological) for the commercial food enzyme preparation that, when met, would render the food enzyme preparation to be of food-grade quality – see section 2.4 (b) of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>
	(b) Data and corresponding certificates of analysis from at least three, non-consecutive batches of the commercial food enzyme preparation or food enzyme concentrate demonstrating the commercial food enzyme preparation or food enzyme concentrate meets the established chemical and microbiological food-grade specifications for the commercial food enzyme preparation

<sup>5</sup> These data should establish the conditions under which the food enzyme is optimally active and conditions under which the food enzyme is inactive (e.g., as a result of denaturation).

<sup>6</sup> If the food enzyme is expected to be denatured in the food(s) in which it is proposed for use, you must include an explanation as to why the processing conditions of that/those food(s) would denature the food enzyme OR a scientific rationale that explains why the food enzyme would not be expected to be active in that/those food(s).

<sup>7</sup> If the food enzyme is expected to be decreased in level due to further processing, you must provide a chemistry-based rationale explaining the steps in the manufacturing processes of that/those food(s) that contribute to the decreased level of the food enzyme.

2.2 Purpose and level of use of the food enzyme	
2.2.1 Purpose	
	(a) Detailed description of the intended technical effect(s) of the food enzyme in the food that is manufactured with the food enzyme <sup>8</sup>
	(b) Exhaustive list of the specific foods or food categories (with a detailed description of each food category and examples of foods captured therein) in which the food enzyme is proposed for use <sup>9</sup>
2.2.2 Proposed maximum level of use	
	(a) Maximum recommended level(s) of use in each specific food or food category <sup>10</sup> in which the food enzyme is proposed for use. The maximum recommended level(s) of use must be expressed in terms of the amount of TOS per amount of raw material.
	(b) Technological justification for the use of the food enzyme in each specific food or food category at the corresponding maximum recommended level of use
2.2.3 Directions for use	
	Directions for use of the food enzyme – see section 2.2.2 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>
2.3 Analytical method and residues	
<b>Note:</b> This information does not need to be included in the initial submission. The Food Directorate will request it if it is needed.	
	(a) A method of analysis for determining the amount of the food enzyme and any substance(s) in the food that results from the use of the food enzyme – see section 2.2.3 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>
	(b) Data to indicate the residues that may remain in or upon the food that is manufactured with the food enzyme when the food enzyme is used in accordance with Good Manufacturing Practice (GMP) <sup>11</sup>
	(c) Proposed maximum limit(s) for residues of the food enzyme in or upon the food that is manufactured with the food enzyme

<sup>8</sup> This description must include a detailed scientific explanation of how the food enzyme exerts its technical effect and why this effect is beneficial.

<sup>9</sup> If the proposed use(s) of the food enzyme impact(s) standardized foods (<https://inspection.canada.ca/food-labels/labelling/industry/standards-of-identity-for-food/eng/1468511768544/1468511932838>), you must indicate the specific standardized food and section from Part B of the *Food and Drug Regulations* ([https://laws-lois.justice.gc.ca/eng/Regulations/c.r.c.,\\_c.\\_870/index.html](https://laws-lois.justice.gc.ca/eng/Regulations/c.r.c.,_c._870/index.html)) that would be impacted by the use of the food enzyme.

<sup>10</sup> In the case of infant formula, the maximum recommended level of use must be expressed per 100 mL and per 100 kcal of infant formula.

<sup>11</sup> Where the limit prescribed for a food additive is stated to be “Good Manufacturing Practice”, the amount of the food additive added to a food in manufacturing and processing shall not exceed the amount required to accomplish the purpose for which the additive is permitted to be added to that food.

2.4 Technological justification	
<b>Note:</b> Include this information in the submission if:	
(1) The food enzyme is not already set out in column 1 in the <a href="#">list of permitted food enzymes</a> , (2) the catalytic activity of the food enzyme is not already permitted in Canada, or (3) the food enzyme is proposed for use in new foods.	
	Data establishing that the food enzyme will have the intended technical effect(s) under the proposed conditions of use <sup>12</sup> – see section 2.2.2 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>

### Section 3: Assessment of potential dietary exposure for the general population

3.1 Dietary exposure for general population	
	A Budget Method estimate of exposure expressed as milligram TOS/kilogram bodyweight per day (calculations must be included) – see section 2.2.4.1 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>

### Section 4: Toxicological safety

**Note:** Please see sections 2.2.4.2 and 2.2.4.3 of [A Guide for the Preparation of Submissions on Food Additives](#) for guidance on the toxicological safety considerations.

4.1 Toxicological studies	
	4.1.1 Short term (e.g., 90 days), oral
	(a) Study follows current Organisation for Economic Cooperation and Development (OECD) test guidelines
	(b) Original study report
	4.1.2 Genotoxicity, mutagenicity
	(a) Study follows current OECD test guidelines
	(b) Original study report
	4.1.3 Genotoxicity, clastogenicity
	(a) Study follows current OECD test guidelines
	(b) Original study report

<sup>12</sup> Ideally, efficacy is demonstrated by the results of tests using graded levels of the food enzyme in the foods of interest under conditions that are representative of the “real world” conditions under which the food enzyme would be expected to function.

4.2 Toxicological safety narrative	
	(a) Detailed discussion of the toxicological safety of the food enzyme under the proposed conditions of use with references to supporting data (e.g., toxicological studies)
	(b) For each study provided, a discussion of how the results of the study support the toxicological safety of the food enzyme under the proposed conditions of use
	(c) Relevance (e.g., similarities/differences) of the test articles used in the toxicology studies to the safety of the food enzyme
4.3 Read-across of toxicological data	
<p>Read-across is a safety assessment approach for food enzymes from microbial sources whereby toxicological safety data for an enzyme produced by one microbial strain can be used to support the safety of an enzyme produced by a closely-related strain.</p> <p><b>Note:</b> If you used this safety-assessment approach, a detailed discussion of the proposed read across approach must be provided, and the following must be included, as applicable, for the Food Directorate to consider the bridging of data:</p>	
	(a) Scientific data showing that the strain used to produce the enzyme concentrate used in the toxicological tests [toxicological production strain (TPS)] and the strain used to produce the enzyme concentrate that is the subject of the submission [requested production strain (RPS)] share a common ancestor, and an overview of how both were developed from the common ancestor
	(b) Scientific data informing on the molecular characterization of each genetic change, including unintended changes, between the TPS and RPS, along with a detailed explanation for why each change is not a concern for safety
	(c) Indication of any mutagenesis steps between the common ancestor and the TPS and RPS, where applicable
	(d) Scientific data supporting a thorough characterization of the mutations between the common ancestor, the TPS, and the RPS as a result of a mutagenesis step
	(e) Table indicating the differences in manufacture between the enzyme from the TPS and the enzyme from the RPS, along with a detailed explanation for why each difference does not impact the toxicological safety of the enzyme from the RPS
	(f) Scientific data demonstrating the safety of the proteins in the enzyme concentrate from the RPS, which are not present in the enzyme concentrate from the TPS

<b>Section 5: Allergenic safety</b>	
<b>5.1 Allergenic safety narrative</b>	
	(a) Detailed discussion of the allergenic safety of the food enzyme under the proposed conditions of use with reference to supporting data
	(b) For each study provided, a discussion of how the results of the study support the allergenic safety of the food enzyme under the proposed conditions of use
	(c) Comments on the presence of <a href="#">priority allergens</a> in the commercial food enzyme preparation

<b>Section 6: Nutritional safety</b>	
<b>6.1 Composition</b>	
	(a) Information on the ability of the food enzyme to reduce or increase the nutrient content of the food manufactured with the food enzyme – see section 2.2.4.4 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>
	(b) Quantitative estimate of how the proposed use of the food enzyme will change the nutrient content of the food manufactured with the food enzyme
<b>6.2 Nutrient bioavailability</b>	
	Information on how the use of the food enzyme and its reaction products enhance or inhibit the bioavailability <sup>13</sup> of nutrients in the food that is manufactured with the food enzyme <sup>14</sup>
<b>6.3 Gastrointestinal effects</b>	
	(a) Information on how the use of the food enzyme and its reaction products affect the gastrointestinal tolerability of the food that is manufactured with the food enzyme
	(b) Detailed discussion on the likelihood and severity of any gastrointestinal effects caused by consumption of food manufactured with the food enzyme, considering 6.3(a)
<b>6.4 Use of the food enzyme in infant formula</b>	
<p>If the food enzyme is intended to be used in infant formula, the following information must be included, as applicable. Otherwise, please provide written confirmation, in the corresponding subsection (i.e., 6.4) of your submission, that the food enzyme is not intended to be used in infant formula.</p> <p><b>Note:</b> Only provide the information required by subsection 6.4(d) if the infant formula is intended for special medical purposes.</p>	

<sup>13</sup> Bioavailability refers to the accessibility of a nutrient to participate in metabolic or physiological processes.

<sup>14</sup> If changes to nutrient bioavailability are identified, you must provide a detailed discussion explaining how these changes may affect metabolic or physiological processes of the population as a whole and/or for specific groups. For example, if the absorption of dietary calcium is enhanced or inhibited by the use of the food enzyme or its reaction products, describe how this may change the health status of a vulnerable population.

	(a) Identification of the specific infant consumption sub-population(s) (e.g., healthy term infants, pre-term infants, infants with medical conditions)
	(b) Comments on the proposed use of the food enzyme in infant formula for special medical purposes
	(c) Maximum proposed level of use of the food enzyme in the infant formula, expressed per 100 mL and per 100 kcal of infant formula
	(d) Consumption rate of the infant formula by each infant sub-population
6.5 Nutritional safety narrative	
	Detailed narrative that supports the overall nutritional safety <sup>15</sup> of the food enzyme and its reaction products under the proposed conditions of use, considering the totality of the evidence

## Section 7: Molecular biological and microbiological safety

### 7.1 General information on the proposed food enzyme

	Information on the history of safe use of the food enzyme and its microbial source organism (species and strain levels), where applicable, in other jurisdictions
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### 7.2 Safety of the source organism

**Note:** Only provide the information required by subsections 7.2(e) through 7.2(g) and 7.2(i) through 7.2(m) if the food enzyme is derived from a microorganism that has been genetically modified or is itself a genetically modified microorganism. Otherwise, please provide written confirmation, in the corresponding subsection (i.e., 7.2) of your submission, that the food enzyme is not derived from a microorganism that has been genetically modified or is itself a genetically modified microorganism.

	(a) Taxonomical name of the source and donor organisms (family, genus, species, and strain) based on the ICNP (International Code of Nomenclature of Prokaryotes) or the International Code of Nomenclature for algae, fungi, and plants
	(b) Deposition/ accession number of the source organism if it has been deposited in an official recognized culture collection
	(c) Comments on the potential of the source and donor organisms for pathogenicity, virulence, or other hazards to human health, such as metabolites, allergens, or antimicrobial resistance genes – see part A of section 2.2.4.5 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>

<sup>15</sup> Nutritional safety includes risks to human health resulting from changes to the nutrient content, nutrient bioavailability, or gastrointestinal tolerability of the food manufactured with the food enzyme under conditions of the proposed use.

	(d) Demonstration that the source organism does not express clinically relevant antibiotics – see part A of section 2.2.4.5 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>
	(e) Where the gene(s) of interest (e.g., coding sequence, signal sequence, codon optimization,) differ(s) from its/their wildtype counterpart, information on the nature of those changes and safety implications
	(f) Information on the design of expression cassette(s) and/or vector(s)
	(g) High level description of the design and development of the production strain
	(h) Information indicating that the production strain is well-characterized morphologically and phenotypically
	(i) Scientific data that establish the molecular characterization of the source organism, including but not limited to insert intactness, expression cassettes copy number, orientation of tandem expression cassettes, integration sites, specificity of integration – see part B of section 2.2.4.5 of: <a href="#">A Guide for the Preparation of Submissions on Food Additives</a>
	(j) Information on any genes and/or regulatory elements that were disrupted, deleted, silenced, modified, or affected by the insertion at the integration site(s), or are proximal <sup>16</sup> to the insertion site(s)
	(k) Scientific data that demonstrate the genetic stability of the integration event or plasmid across several generations
	(l) Information on whether the integration event(s) is/are likely to affect expression of genes proximal to the insertion site(s)
	(m) Bioinformatic analysis performed at the insertion site(s) and flanking sequences for any similarity hits against known toxins, allergens, and secondary metabolites

<sup>16</sup> Proximal refers to regions immediately flanking the insertion site.

## Section 8: Additional considerations

**Note:** While the following information is not mandatory at this time, providing it may facilitate the evaluation process.

### 8.1 Authorization of food enzymes in other jurisdictions and Codex Alimentarius provision(s)

Indicate whether the food enzyme is permitted or is under review in Australia and New Zealand, Europe, and the United States, for the same use(s) that you have requested in Canada, and whether there are provisions within any international Codex Alimentarius food standards, including the *General Standard for Food Additives*, that would accommodate such use(s), or provisions that are under consideration by Codex. Provide the documentation that substantiates the authorization or provision [e.g., letter from regulatory authority; reference to regulation; reference to provision in Codex standard(s)].

### 8.2 Domestic Substances List (DSL) and *New Substances Notification Regulations* (NSNR)

(a) Indicate whether the food enzyme and its source are listed on the [Domestic Substances List](#)

(b) Explain whether notification of the food enzyme under the [New Substances Notification Regulations](#) is required

## Section 9: Proposed food additive label

**Note:** This information does not need to be included in the initial submission. The Food Directorate will request it if it is needed.

Specimens of the labelling proposed for the commercial food enzyme preparation – see section 2.2.5 of: [A Guide for the Preparation of Submissions on Food Additives](#)