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Health Canada's Proposal to Enable the Use of Glutaminase from *Bacillus amyloliquefaciens* GT2 as a Food Enzyme in various unstandardized foods

Notice of Proposal – *Lists of Permitted Food Additives*

Reference Number: [NOP/ADP-0031]

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Bureau of Chemical Safety
Food Directorate
Health Products and Food Branch

Canada

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Summary

Food additives are regulated in Canada under Marketing Authorizations (MAs) issued by the Minister of Health and the *Food and Drug Regulations* (Regulations). Approved food additives and their permitted conditions of use are set out in the Lists of Permitted Food Additives that are incorporated by reference in the MAs and published on the Canada.ca website. A petitioner can request that Health Canada approve a new additive or a new condition of use for an already approved food additive by filing a food additive submission with the Department's Food Directorate. Health Canada uses this premarket approval process to determine whether the scientific data support the safety of food additives when used under specified conditions in foods sold in Canada.

Health Canada's Food Directorate received a food additive submission seeking approval for the use of the enzyme glutaminase from *Bacillus amyloliquefaciens* GT2 in dairy-based flavouring preparations and other unstandardized flavouring preparations; yeast extract; hydrolyzed animal, milk and vegetable protein; and standardized egg products. The enzyme is intended to be used, at a level consistent with Good Manufacturing Practice (GMP), to increase the level of glutamic acid in the foods of interest in order to enhance the desired umami flavour, similar to the flavour achieved by using monosodium glutamate (MSG).

The Food Directorate concluded that information related to the safety and efficacy of glutaminase from *B. amyloliquefaciens* GT2 supports its use as requested by the petitioner, except that efficacy data were provided for one type of dairy-based flavouring preparation and not for unstandardized flavouring preparations in general. Also, although the petitioner had requested that glutaminase be permitted for use in standardized egg products, Health Canada and the Canadian Food Inspection Agency (CFIA) collectively determined that egg products treated with glutaminase should be considered unstandardized. Therefore, Health Canada proposes to enable the use of glutaminase from *B. amyloliquefaciens* GT2 in the foods shown in the table below by adding the entries in the table to the *List of Permitted Food Enzymes*.

Proposed Modification to the *List of Permitted Food Enzymes*

Item No.	Column 1 Additive	Column 2 Permitted Source	Column 3 Permitted in or Upon	Column 4 Maximum Level of Use and Other Conditions
G.5	Glutaminase	*Bacillus amyloliquefaciens* GT2	(1) Dairy-based flavouring preparations	(1) Good Manufacturing Practice
			(2) Hydrolyzed animal, milk and vegetable protein	(2) Good Manufacturing Practice

Item No.	Column 1 Additive	Column 2 Permitted Source	Column 3 Permitted in or Upon	Column 4 Maximum Level of Use and Other Conditions
			(3) Unstandardized egg products	(3) Good Manufacturing Practice
			(4) Yeast Extract	(4) Good Manufacturing Practice

Rationale

The Food Directorate completed a premarket safety and efficacy assessment of the requested uses of glutaminase from *B. amyliloquefaciens* GT2. The assessment concluded that information related to chemistry, efficacy, toxicology, allergenicity, nutrition, molecular biology and microbiology supports the safety of glutaminase from *B. amyliloquefaciens* GT2 for its requested uses.

Glutaminase catalyzes the hydrolysis of L-glutamine to L-glutamate and ammonia.[1] Data were provided showing that glutaminase from *B. amyliloquefaciens* GT2 exhibits activity between pH 3.5 and 9.5 and between 37°C and 70°C, with optimal pH and temperature being pH 6.0-7.0 and 60-70°C. The enzyme is shown to be inactivated with heat treatment above 70°C and, since the foods of interest are subject to such heat treatment during their manufacturing process, little or no residual glutaminase activity is expected in the final foods. The results of testing demonstrated that glutaminase from *B. amyloliquefaciens* GT2 is effective in increasing the level of glutamic acid in dairy-based flavouring preparations; yeast extract; hydrolyzed animal, milk and vegetable protein; and unstandardized egg products.

The results of standard toxicological tests with glutaminase from *B. amyliloquefaciens* GT2 support that this enzyme is safe for its proposed uses in food. There is no significant sequence homology between the glutaminase enzyme's amino acid sequence and known allergens, and the amounts of L-glutamate and ammonia that will be present in foods as a result of the reaction catalysed by glutaminase do not present a food safety concern.

Intake of glutaminase enzyme protein that remains in finished foods will be insignificant relative to the total dietary intake of protein, and the sodium chloride used in the commercial glutaminase enzyme preparation will not contribute significantly to total sodium intake in the Canadian diet.

The production organism *B. amyliloquefaciens* GT2 is an isolate from the parent strain *B. amyloliquefaciens* NP. The parent strain has an extensive history of safe use as a source organism for food products and processing aids around the world. The production strain GT2 is expected to be equally safe from a microbial food safety perspective.

[1] Hydrolysis reaction for EC 3.5.1.2 (glutaminase) as stated in the IUMBM (https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/5/1/2.html)

The results of the premarket assessment support the safety of glutaminase from *B. amyliloquefaciens* GT2 for use as a food enzyme as set out in the table above. Health Canada is therefore proposing to enable the use of this food additive as shown in the table.

Other Relevant Information

Glutaminase will increase the free glutamic acid content of the foods it is added to. When glutamic acid or its salts (e.g. MSG) or hydrolyzed plant protein are added to a prepackaged food, they must be declared in the list of ingredients on the food label, even if they are a component of a preparation or mixture such as flavouring preparation or seasoning mixture that is usually exempt from component declaration.[2] This labeling is not required for other food ingredients or foods that contain free glutamate. However, the Food Directorate provides educational information in this regard for consumers at a questions and answers website about MSG[3] and intends to update the website to include information about glutaminase.

In Australia and New Zealand, food enzymes are regulated as processing aids under the *Australia New Zealand Food Standards Code* (the Code) and are subject to premarket approval. Food Standards Australia New Zealand (FSANZ) completed an assessment of glutaminase from *B. amyloliquefaciens* GT2 for use in the production of certain seasoning ingredients (e.g. yeast extracts, hydrolyzed vegetable protein, hydrolyzed animal protein) and food products used as seasoning (e.g. soy sauce, miso, vinegar, fish sauce).[4] FSANZ did not identify any public health or safety issues with the proposed uses and the enzyme and source organism are listed in Schedule 18 of the Code.

Currently in the European Union (EU), the regulation of food enzymes is subject to each EU country's legislation.[5] In France, the federal agency that oversees food safety did not identify any health risks associated with the use of glutaminase from *B. amyloliquefaciens* GT2 to produce protein hydrolysate and yeast extract.[6]

Amano Enzyme has not submitted a Generally Recognized as Safe (GRAS) notification for glutaminase to the United States Food and Drug Administration.

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[2] This requirement is set out in section B.01.009 of the *Food and Drug Regulations*

[3] Monosodium glutamate (MSG) – Questions and Answers https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/monosodium-glutamate-questions-answers.html

[4] See FSANZ website "A1109 – Glutaminase from Bacillus amyloliquefaciens as a Processing Aid (Enzyme)". http://www.foodstandards.gov.au/code/applications/Pages/A1109Glutaminase.aspx

[5] EU List and Applications. https://ec.europa.eu/food/safety/food_improvement_agents/enzymes/eu_list_app_en

[6] Avis de l'Agence française de sécurité sanitaire des aliments relatif à une demande d'autorisation d'emploi sous deux formulations d'une glutaminase produite par une souche de *Bacillus amyloliquefaciens* non modifiée génétiquement pour la production d'hydrolysats de proté.ines et d'extraits de levures. Agence Française de Sécurité Sanitaire des Aliments, Maisons-Alfort, le 16 juillet 2009. https://www.anses.fr/en/system/files/BIOT2009sa0089.pdf

The Canadian *Food and Drug Regulations* require that food additives such as glutaminase from *B. amyloliquefaciens* GT2 that do not have food-grade specifications set out in Part B of the Regulations meet the most recent food-grade specifications set out in the *Food Chemicals Codex* or the *Combined Compendium of Food Additive Specifications*. The *Food Chemicals Codex* is a compendium of standards for purity and identity for food ingredients, including food additives, published by the United States Pharmacopeial Convention. The *Combined Compendium of Food Additive Specifications* and its associated *General Specifications and Considerations for Enzyme Preparations* are both prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and published by the Food and Agriculture Organization of the United Nations.

Implementation and Enforcement

The proposed change will be effective the day on which it is published in the *List of Permitted Food Enzymes*. This will be announced via a Notice of Modification that will be published on the Government of Canada's Website.

The CFIA is responsible for the enforcement of the *Food and Drugs Act* and its associated regulations with respect to foods.

Contact Information

For additional information or to submit comments related to this proposal, please contact:

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If communicating by e-mail, please use the words "glutaminase **(NOP-0031)**" in the subject line of your e-mail. Health Canada is able to consider information received by **April 20, 2019**, 75 days from the date of this posting.