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Health Canada's Proposal to Enable the Use of Protein-glutaminase from *Chryseobacterium proteolyticum* AE-PG as a Food Enzyme in Various Standardized and Unstandardized Foods

Notice of Proposal – *Lists of Permitted Food Additives*

Reference Number: NOP/ADP-0030

October 10, 2018

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 protein-glutaminase from *Chryseobacterium proteolyticum* AE-PG. The petitioner requested its use in bread; flour; whole wheat flour; hydrolyzed animal, milk and vegetable protein; pasta¹; plant-based beverages such as soy beverage or oat beverage; unstandardized bakery products; unstandardized dairy products such as yogurt, frozen desserts, and dairy-based beverages; and yeast extract. The requested maximum level of use for the enzyme is Good Manufacturing Practice (GMP).

The technical effect(s) achieved through the use of protein-glutaminase is based in part on the enzyme's ability to increase the water solubility of food protein.

The results of Health Canada's evaluation of the available scientific data support the safety and efficacy of protein-glutaminase from *C. proteolyticum* AE-PG when used as requested by the petitioner. Therefore, Health Canada intends to enable its requested uses by adding the entries shown in the table below 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
P.6.1	Protein-glutaminase	<i>Chryseobacterium proteolyticum</i> AE-PG	(1) Bread; Flour; Whole wheat flour	(1) Good Manufacturing Practice
			(2) Hydrolyzed animal, milk and vegetable protein	(2) Good Manufacturing Practice
			(3)	(3)

¹ Pasta is considered a synonym for "alimentary paste", as described in Division 13, Section B, of the Regulations. Pasta (alimentary paste) includes noodles, macaroni, spaghetti, etc.

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Item No.	Column 1 Additive	Column 2 Permitted Source	Column 3 Permitted in or Upon	Column 4 Maximum Level of Use and Other Conditions
			Pasta	Good Manufacturing Practice
			(4) Plant-based beverages	(4) Good Manufacturing Practice
			(5) Unstandardized bakery products	(5) Good Manufacturing Practice
			(6) Unstandardized dairy products	(6) Good Manufacturing Practice
			(7) Yeast extract	(7) Good Manufacturing Practice

Rationale

The Food Directorate completed a premarket safety and efficacy assessment of protein-glutaminase from *C. proteolyticum* AE-PG for use as a food enzyme. The assessment considered microbiological, toxicological and food allergy, nutritional, chemical and technical aspects of protein-glutaminase from *C. proteolyticum* AE-PG when used as requested in the food additive submission.

Protein-glutaminase is identified under the IUBMB² system of nomenclature as “protein-glutamine glutaminase”. The enzyme catalyzes the hydrolysis of the amide functional group of protein-bound glutamyl residues, releasing an ammonia molecule in the process. A carboxyl group takes the place of the amide functional group. The change in functional group is expected to cause the protein in food to be more soluble, which in turn should result in one or more different technical effects depending on the final food. Food protein otherwise remains intact. No free amino acids, including free glutamic acid, are produced as a result of the enzymatic reaction.

Protein-glutaminase from *C. proteolyticum* AE-PG exhibits activity between pH 3 and 10 and between 20°C and 70°C, with optimal pH and temperature being pH 5.0-7.0 and 50-60°C. The enzyme is inactivated at temperatures above 70°C.

No toxicological food safety concerns were identified from the results of standard toxicological tests with protein-glutaminase from *C. proteolyticum* AE-PG. The enzyme’s amino acid sequence does not match known allergens.

² IUBMB is the International Union of Biochemistry and Molecular Biology.

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The evaluation identified no concerns from a nutritional perspective. The amount of protein-glutaminase protein remaining in finished foods would be insignificant relative to the total dietary intake of protein.

The bacterium that is used to produce the enzyme (*C. proteolyticum* AE-PG) was derived using classical mutagenesis. It is a microbiologically safe and suitable source for food enzyme production.

The results of the premarket assessment support the safety of protein-glutaminase from *C. proteolyticum* AE-PG for use as a food enzyme as set out in the table above. Health Canada is therefore proposing to enable the uses of this food enzyme as shown in the table.

Other Relevant Information

The United States Food and Drug Administration (FDA) had no questions in response to a self-affirmed GRAS notification for the use of protein-glutaminase enzyme preparation from *C. proteolyticum* (strain 9670) in vegetable proteins (cereal, soy, and pea), milk proteins, yeast proteins; baked goods; dairy foods such as yogurt, cheese, and ice-cream; egg-based foods such as mayonnaise, and cakes.³ This strain is the same production organism as *C. proteolyticum* AE-PG.⁴

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 pre-market approval. Food Standards Australia New Zealand (FSANZ) completed an assessment of protein-glutaminase from *C. proteolyticum* for use in food categories that are comparable to those covered by the submission to Health Canada.⁵ FSANZ did not identify any public health or safety issues, and also concluded that the proposed uses were technologically justified. However, protein-glutaminase from this source is not currently permitted as it has not yet been added to the Code.

The European Union (EU) is working toward the establishment of a list of permitted food enzymes. In this regard, an application to include “protein-glutamine glutaminase” (synonymous with protein-glutaminase) from *C. proteolyticum* AE-PG in the pending EU list of authorized food enzymes was submitted to the European Commission for consideration.⁶ The enzyme is to

³ Agency Response Letter GRAS Notice No. GRN 000267. Letter of July 15, 2009, from L.M. Tarantino to J.B. Dubeck. Available at the following website: <https://wayback.archive-it.org/7993/20171031030733/https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm174944.htm> (Accessed October 2018.)

⁴ Yamaguchi, S. and Yokoe, M., 2000. A Novel Protein-Deamidating Enzyme from *Chryseobacterium proteolyticum* sp. Nov., a Newly Isolated Bacterium from Soil. *Appl. Environ. Microbiol.*, 66(8), 3337-3343.

⁵ Supporting document 1. Risk and technical assessment – Application A1136. Protein Glutaminase as a Processing Aid (Enzyme). 22 February 2018 [39-18]. Food Standards Australia New Zealand.

⁶ Food enzyme applications submitted to the Commission within the legal deadline (from 11 September 2011 to 11 March 2015). Version 4. Updated on 25 July 2016. European Commission Directorate-General for Health and Food

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be used in food categories that are comparable to those covered by the submission to Health Canada.

The *Food and Drug Regulations* require that food additives such as protein-glutaminase from *C. proteolyticum* AE-PG 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 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 Canadian Food Inspection Agency 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 “**protein-glutaminase (NOP-0030)**” in the subject line of your e-mail. Health Canada is able to consider information received by **December 23, 2018**, 75 days from the date of this posting.