U.S. Food and Drug Administration
Department of Health and Human Services
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

CFSAN/Office of Food Additive Safety
November 24, 2006

Agency Response Letter
GRAS Notice No. GRN 000201

Ms. Lori Gregg
Novozymes North America, Inc.
77 Perry Chapel Church Road
P.O. Box 576 Franklinton, NC 27525

Re: GRAS Notice No. GRN 000201

Dear Ms. Gregg:

The Food and Drug Administration (FDA) is responding to the notice, dated May 30, 2006, that you submitted in accordance with the agency's proposed regulation, proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS); the GRAS proposal). FDA received the notice on June 1, 2006, filed it on June 5, 2006, and designated it as GRAS Notice No. GRN 000201. Novozymes responded to questions from FDA in amendments dated July 27, 2006, and August 5, 2006.

The subject of the notice is asparaginase enzyme preparation from *Aspergillus oryzae* expressing a gene encoding an asparaginase from *A. oryzae* (*A. oryzae* asparaginase enzyme preparation). The notice informs FDA of the view of Novozymes North America, Inc (Novozymes) that *A. oryzae* asparaginase enzyme preparation is GRAS, through scientific procedures, for use in reducing asparagine levels in wheat dough-based products such as cookies and crackers, fabricated potato chips, and cut or sliced potato products.[1]

Novozymes provides general information about the identity and technical effect of asparaginases as well as specific information about the identity and activity of the asparaginase enzyme preparation that is the subject of GRN 000201. It is identified by IUB name L-asparagine amidohydrolase, EC No. 3.5.1.1, and Chemical Abstracts Services (CAS) Registration No. 9015-68-3. Asparaginase hydrolyzes the amide of asparagine to form aspartic acid and ammonia. The amino acid sequence of this asparaginase and the nucleotide sequence of the gene encoding it have been determined. Novozymes notes that asparaginase also hydrolyzes glutamine, but not other free amino acids or asparagine residues within peptides or proteins. Novozymes characterizes the asparaginase in its *A. oryzae* asparaginase enzyme preparation by its molecular weight (approximately 36,000 Daltons (Da)), temperature and pH optima (60 degrees Celsius and 7.0, respectively), and activity range (pH 5.0 to 9.0).

Novozymes notes that a variety of bacteria and fungi produce asparaginases in cytoplasmic, periplasmic, or extracellular forms. Novozymes notes that extracellular asparaginases, such as the *A. oryzae* asparaginase, have higher affinity for asparagine than cytoplasmic forms.

The production strain was bioengineered to produce asparaginase under the control of the neutral amylase promoter of *A. niger*. Novozymes considers that an enzyme preparation derived from a recombinant microorganism will be safe if the host microorganism is nontoxigenic and nonpathogenic, the genetic information that is introduced into the host microorganism is well characterized, and the added DNA does not encode and express any known harmful or toxic substances. The framework for this conclusion is based on published comprehensive reviews and reports.

Novozymes discusses that the recipient strain *A. oryzae* BECh2 is derived from a safe lineage including strains that Novozymes has used for enzyme production for over 30 years. Novozymes published safety studies on two products from *A. oryzae* strains developed from *A. oryzae* A1560, the parent of BECh2. The recipient strain was used to construct production strains for modified lipase, glucose oxidase, and phospholipase enzyme preparations (the subjects of GRN Nos. 000103, 000106, and 000142, respectively). Novozymes notes that the changes made in BECh2 are well characterized, specific, and do not encode for elements that might adversely affect safety.

Novozymes describes the genes on its asparaginase expression plasmid, similar in construction to expression plasmids used to construct production strains for lipase (GRN 000103) and phospholipase A1 (GRN 000142), and describes the differences among these expression plasmids. Novozymes states that the DNA is randomly integrated into the chromosome, and that the production strain is well characterized by qualified scientists and technicians with sufficient expertise in identifying and characterizing strains to prevent contamination and ensure acceptable yields of a functional enzyme product. Novozymes considers its monitoring sufficient to detect unexpected secondary effects from genetic modifications.

Novozymes states that the method of manufacture for its asparaginase enzyme follows standard industry practices, complies with requirements of ISO 9001, and is in accordance with current good manufacturing practices. Novozymes describes raw materials used in fermentation and recovery for its asparaginase enzyme preparation as standard ingredients in the enzyme industry, conforming either to the Food Chemicals Codex (FCC) specifications or to internal specifications consistent with FCC requirements. Novozymes' quality control department ensures that raw materials meet specifications.

Novozymes describes asparaginase enzyme preparation as produced under submerged fed-batch pure culture of the production strain with equipment designed, operated, and cleaned to prevent microbial contamination. Novozymes recovers their asparaginase from culture broth by pH adjustment, primary filtration, concentration by ultrafiltration or evaporation, followed by prefiltration and germ filtration to remove residual microorganisms. The fluid concentrate is then preserved, stabilized, and then further evaporated or ultrafiltered as needed to achieve the appropriate activity. Stabilized concentrate is blended with glycerol and water and preserved with sodium benzoate and potassium sorbate.

Novozymes provides specifications for the asparaginase enzyme preparation, noting total organic solids (TOS) at approximately four percent, with the remainder as glycerol, water,

sodium benzoate, and potassium sorbate at 50, 46, 0.3 and 0.1 percent, respectively. Novozymes states that the specifications for its asparaginase enzyme preparation comply with purity criteria for enzyme preparations in the FCC and with General Specifications for Enzyme Preparations used in Food Processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in the Compendium of Food Additive Specifications.

Novozymes states that dough-based applications would use from 200-2500 ASNU[2] per kg processed food, corresponding to 6-70 g of asparaginase preparation per 100 kg processed food. Cut vegetables would use approximately 200 ASNU per kg final product, reflecting treatment in enzyme baths designed to pick up approximately 600 ASNU per kg treated potatoes.

Although Novozymes acknowledges that asparaginase would be heat inactivated by food processing, Novozymes calculates exposure (as estimated daily intake, EDI) assuming that added enzyme would be retained in the final products. Novozymes presumed that all processed food products (half of all food intake) would use the enzyme preparation at the highest recommended usage level and that all TOS would remain in the final product. Novozymes calculated an EDI of 0.35 mg TOS per kilogram body weight per day.

Novozymes includes results from unpublished studies; an *in vitro* bacterial reverse mutation assay with and without metabolic (S9) activation and an *in vitro* cytotoxicity test to conclude that the test preparation (a liquid asparaginase enzyme concentrate prepared like the commercial preparation, but without stabilization and standardization) is nonmutagenic and noncytoxic. Novozymes also discusses results from published and unpublished 13 week oral toxicity studies of enzyme preparations from *A. oryzae* strains, emphasizing studies using enzymes produced from strains derived from strain BECh2 or its parent A1560. These studies concluded that test preparations did not exhibit toxicity or mutagenicity under the conditions of the tests. Novozymes concludes that these studies support the safe use of enzyme preparations produced by strains derived from *A. oryzae* BECh2.

Based on the information provided by Novozymes, as well as other information available to FDA, the agency has no questions at this time regarding Novozymes' conclusion that *A. oryzae* asparaginase enzyme preparation is GRAS under the intended conditions of use. The agency has not, however, made its own determination regarding the GRAS status of the subject use of *A. oryzae* asparaginase enzyme preparation. As always, it is the continuing responsibility of Novozymes to ensure that food ingredients that the firm markets are safe, and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with proposed 21 CFR 170.36(f), a copy of the text of this letter responding to GRN 000201, as well as a copy of the information in this notice that conforms to the information in the proposed GRAS exemption claim (proposed 21 CFR 170.36(c)(1)), is available for public review and copying on the homepage of the Office of Food Additive Safety (on the Internet at http://www.cfsan.fda.gov/~lrd/foodadd.html).

Sincerely,

Laura M. Tarantino, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

(1)Novozymes describes the intended effect of the asparaginase as the conversion of asparagine to aspartic acid to reduce the formation of acrylamide in specified products. FDA neither evaluated the efficacy of such treatments nor determined whether acrylamide levels detected by Novozymes in untreated foods represent a significant health concern.

(2)One ASNU (asparaginase unit) produces one micromole ammonia per minute under specific reaction conditions.

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