



Emily Gregoire  
Chr. Hansen, Inc.  
9015 West Maple Street  
Milwaukee, WI 53214-4298

Re: GRAS Notice No. GRN 000801

Dear Ms. Gregoire:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 000801. We received Chr. Hansen, Inc. (Chr. Hansen)'s GRAS notice on July 13, 2018 and filed it on August 16, 2018. We received amendments containing additional safety information on March 7, 2019, March 29, 2019, May 21, 2019, and June 5, 2019.<sup>1</sup>

The subject of the notice is chymosin enzyme preparation produced by *Aspergillus niger* expressing a modified gene encoding a protein engineered variant of chymosin from *Camelus dromedarius* (chymosin enzyme preparation) for use as an enzyme at up to 1.2 mg Total Organic Solids (TOS)/L of milk during the production of cheese and whey. The notice informs us of Chr. Hansen's view that this use of chymosin enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing typically contain an enzyme component that catalyzes the chemical reaction as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Chr. Hansen's notice provides information about the components in the chymosin enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, chymosin is identified by the Enzyme Commission Number 3.4.23.4. The accepted name and the systematic name for this enzyme is chymosin. The enzyme is also known as rennin. Chymosin cleaves the Phe105-Met106 bond of  $\kappa$ -casein. The CAS No. for chymosin is 9001-98-3. Chr. Hansen states that the chymosin is 232 amino acids in length with a corresponding molecular weight of 35.5 kDa.

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<sup>1</sup>The March 7, 2019 amendment included information that Chr. Hansen designated confidential. The May 21, 2019 amendment included a revised version of the March 7, 2019 amendment correcting the information designated confidential. In the June 5, 2019 amendment the notifier explained how experts could get to a conclusion of safety without the confidential information.

Chr. Hansen states that the *A. niger* production strain DSM3205<sup>2</sup> was derived from the *A. niger* recipient strain CH-Asp1-TC-4. Chr. Hansen states that this recipient strain was produced by spontaneous mutagenesis and chosen based on reduced proteolytic activity. Chr. Hansen describes *A. niger* as a non-pathogenic, non-toxicogenic, well-characterized production organism with a history of safe use in the food industry.

Chr. Hansen describes the construction of the *A. niger* production strain by targeted integration of an expression cassette carrying a synthetic gene engineered to express a recombinant protein chymosin from *C. dromedarius* fused to an *A. niger* glucoamylase, under control of a native *A. niger* promoter and terminator. The chymosin sequence was modified to increase activity and to ensure native protein folding. Chr. Hansen states that the plasmid also carries a gene encoding a *Neurospora crassa* orotidine 5-phosphate decarboxylase, which is used for selection of transformants. Chr. Hansen states that the stability of the introduced DNA has been confirmed by Southern blot analysis. Chr. Hansen also states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

Chr. Hansen states that the enzyme is produced by submerged fed-batch fermentation of a pure culture of the production strain. Chr. Hansen states that fermentation is carried out under controlled conditions and that the enzyme is secreted as a glucoamylase-chymosin fusion protein into the fermentation broth; the chymosin is autocatalytically released from the glucoamylase-chymosin fusion protein after secretion. After the fermentation is stopped by treatment with acid, chymosin is recovered from filtering the broth; it is separated from glucoamylase by column chromatography, standardized and filtered. The enzyme concentrate is used for the toxicological studies discussed in this notice. It is formulated to the chymosin enzyme preparation with food-grade sodium chloride, sodium benzoate, and water, and is filtered. Chr. Hansen states that the entire process is performed in accordance with current good manufacturing practices. Chr. Hansen also states that the final chymosin enzyme preparation does not contain any major food allergens from the fermentation media.

Chr. Hansen has established food grade specifications and states that the chymosin enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10th edition, 2016), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). Chr. Hansen provides analytical data from three batches of chymosin enzyme concentrate to demonstrate that it can be manufactured to meet these specifications, including the absence of the production strain.

Chr. Hansen intends to use chymosin enzyme preparation in the production of cheese and whey at a maximum level corresponding to 1.2 mg TOS/L of milk. Chr. Hansen notes that the chymosin enzyme preparation will be deactivated or removed during the production process. However, in estimating dietary exposure Chr. Hansen assumes that

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<sup>2</sup> Chr. Hansen states that the production strain, *A. niger* strain Tiegh, is deposited in the Deutsche Sammlung von Mikroorganismen in Germany as DSM 32805.

all the chymosin enzyme preparation will remain in the final food. Chr. Hansen estimates dietary exposure from all uses of chymosin enzyme preparation to be 0.032 mg TOS/kg body weight per day (mg TOS/kg bw/d).<sup>3</sup>

Chr. Hansen relies on published information that discusses the safety of microbial enzyme preparations used in food processing, including the safety of the *A. niger* production organism. Additionally, Chr. Hansen summarizes unpublished toxicological studies using chymosin enzyme liquid concentrate to corroborate safety of the intended uses. These include bacterial reverse mutation assay, *in vitro* micronucleus assay, and a rat 14-day oral toxicity study. Chr. Hansen also discusses the results from an unpublished 13-week oral toxicity study conducted in rats using the non-protein engineered variant of the chymosin enzyme that did not show any treatment-related adverse effects up to the highest dose tested, equivalent to 24.2 mg TOS/kg bw/d.

Chr. Hansen discusses publicly available literature as well as the conclusions of several organizations and working groups about the low risk of allergenicity posed by enzymes to address potential allergenicity due to chymosin. Further, based on bioinformatic analyses, Chr. Hansen reports that the protein engineered variant of chymosin does not share any biologically meaningful sequence homology or sequence identity to toxins and potential oral allergens.<sup>4</sup> Based on the totality of the information available, Chr. Hansen concludes that it is unlikely that oral consumption of chymosin enzyme will result in allergenic responses.

Chr. Hansen includes the report of a panel of individuals (Chr. Hansen's GRAS panel). Based on its review, Chr. Hansen's GRAS panel concluded that chymosin enzyme preparation is safe under the conditions of its intended use.

Based on the data and information summarized above, Chr. Hansen concludes that chymosin enzyme preparation is GRAS for its intended use.

### **Standards of Identity**

In the notice, Chr. Hansen states its intention to use chymosin enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

### **Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)**

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of

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<sup>3</sup> Chr. Hansen calculates estimated dietary exposure to chymosin enzyme preparation based on consumption of a maximum of 38.5 g and 2.9 g of cheese and whey per person per day.

<sup>4</sup> Chr. Hansen identified homology to one fungal protein (aspartyl endopeptidase) during bioinformatic searches but concluded that is an aeroallergen and not likely to cause an allergenic response from consumption.

the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll)(1)-(4) applies. In our evaluation of Chr. Hansen's notice concluding that chymosin enzyme preparation is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing chymosin enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing chymosin enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

## Conclusions

Based on the information that Chr. Hansen provided, as well as other information available to FDA, we have no questions at this time regarding Chr. Hansen's conclusion that chymosin enzyme preparation produced by *A. niger* expressing a modified gene encoding a protein engineered variant of chymosin from *C. dromedarius* is GRAS under its intended conditions of use. This letter is not an affirmation that chymosin enzyme preparation produced by *A. niger* expressing a modified gene encoding a protein engineered variant of chymosin from *C. dromedarius* is GRAS under 21 CFR 170.35. Unless noted above, our review did not address other provisions of the FD&C Act. Food ingredient manufacturers and food producers are responsible for ensuring that marketed products are safe and compliant with all applicable legal and regulatory requirements.

In accordance with 21 CFR 170.275(b)(2), the text of this letter responding to GRN 000801 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,

Susan J.

Carlson -S

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Susan Carlson, Ph.D.

Director

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety

and Applied Nutrition