U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Manki Ho
Chr. Hansen A/S
Boege Allé 10-12
Hoersholm 2970
DENMARK

Re: GRAS Notice No. GRN 001195

Dear Dr. Ho:

This letter corrects our response letter to GRN 001195 signed on March 6, 2025. The purpose of this revised letter is to correct an intended use to "whey production" from "whey powder production," include the units for the dietary exposure estimate of "0.38 mg TOS/kg bw/day" from "0.38 TOS/kg bw/day," and to correct the header to "Dr. Ho."

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001195. We received Chr. Hansen A/S (Chr. Hansen)'s GRAS notice on May 23, 2024 and filed it on September 10, 2024. Chr. Hansen submitted amendments to the notice on January 17, 2025 and February 14, 2025, containing additional information on enzyme identity, enzyme safety narrative, and specifications.

The subject of the notice is chymosin enzyme preparation produced by *Aspergillus niger* expressing a gene encoding chymosin from *Erinaceus europaeus* (European hedgehog; chymosin enzyme preparation) for use as an enzyme at up to 0.97 mg Total Organic Solids (TOS)/kg milk in cheese and whey production. 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,[1] and the Chemical Abstracts Service Number 9001-98-3.

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/4/23/4.html

MCC states that the primary amino acid sequence of the chymosin consists of 332 amino acids with a molecular weight of 35.4 kDa.

Chr. Hansen states that the *A. niger* production organism is a non-pathogenic and non-toxigenic fungus with a history of safe use in food production.
Chr. Hansen states that the *A. niger* production strain "DSM 34579" was constructed from the host strain by targeted integration of an expression cassette carrying a chymosin gene from *E. europaeus* under control of host promoter, terminator and a selectable marker. Chr. Hansen states that whole genome sequencing was used to confirm the sequence integrity of the production strain. Chr. Hansen states that the final production strain does not contain any functional or transferable antibiotic resistance genes.

Chr. Hansen states that chymosin enzyme preparation is manufactured by controlled fermentation of a pure culture of the *A. niger* production strain. The enzyme is secreted into the fermentation medium. After fermentation, the medium containing the enzyme is separated from the biomass, recovered, and concentrated by a series of filtration, column chromatography and elution steps. The resulting chymosin enzyme concentrate is colorless to amber and is formulated to an enzyme preparation, for example to a liquid formulation with the addition of sodium chloride and water. Chr. Hansen states that the entire process is performed in accordance with current Good Manufacturing Practices and with food-grade raw materials. Chr. Hansen also states that the chymosin enzyme preparation does not contain any major food allergens.

Chr. Hansen has established food-grade specifications including a limit for lead (< 0.5 mg/kg) and states that the chymosin enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 13th edition, 2022), 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 results from analyses of three non-consecutive batches of chymosin enzyme concentrate to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production organism in the final product.

Chr. Hansen intends to use chymosin enzyme preparation at a maximum level 0.97 mg TOS/kg milk in cheese and whey powder production to catalyze the hydrolysis of a single peptide bond in κ-casein. Chr. Hansen notes that the chymosin enzyme is inactivated during food production. Chr. Hansen estimates a maximum dietary exposure to the chymosin enzyme preparation to be 0.38 mg TOS/kg bw/day from the use in food and drinks with the assumption that the added chymosin enzyme preparation remains present in the final food.[2]

Chr. Hansen relies on published information that discusses the safety of the *A. niger* production organism, the safety of microbial enzyme preparations used in food

[2] Chr. Hansen uses the Budget method to estimate the dietary exposure to chymosin enzyme preparation based on the consumption of 12.5 g/kg bw/d and 25 mL/kg bw/d containing the chymosin enzyme preparation at the recommended use level.

processing, the safety of the *E. europaeus* donor organism, and the safety of chymosin enzymes. Chr. Hansen summarizes the available published literature that supports the history of safe use of chymosin in food. In addition, Chr. Hansen states that enzymes are generally added at the lowest level to catalyze the desired reaction and that exposure to the consumer is generally low. In support of the safety of chymosin enzyme preparation, Chr. Hansen discusses the high degree of similarity between the sequences of the chymosin and the chymosin that was the subject of GRN 000801, including the catalytic site, and concludes that the notified chymosin enzyme preparation has a similar safety profile to the subject of GRN 000801;[3] thus Chr. Hansen states that the toxicological studies discussed in GRN 000801 can be used to support safety of *E. europaeus* chymosin produced similarly. Also, Chr. Hansen states that a literature search did not identify any information that would contradict a general recognition of safety of the chymosin enzyme preparation.

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 from their intended use, to address potential allergenicity due to chymosin. Based on bioinformatic analyses, using criteria recommended by FAO/WHO (FAO/WHO, 2001; Codex Alimentarius, 2009; JECFA, 2016), Chr. Hansen reports that no sequence homology of chymosin to known allergens that would raise allergenicity concerns were identified. Based on the totality of the information available, Chr. Hansen concludes that it is unlikely that oral consumption of chymosin will result in allergenic responses from its intended uses.

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 CFR. 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 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

[3] GRN 000801 describes the intended food uses of chymosin enzyme preparation. We evaluated GRN 000801 and responded with a letter on June 6, 2010, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

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 is GRAS under its intended conditions of use. This letter is not an affirmation that chymosin enzyme preparation 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 001195 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
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Susan Carlson, Ph.D.
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