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 001201

Dear Dr. Ho:

This letter corrects our response letter to GRN 001201 signed on March 10, 2025. The purpose of this revised letter is to correct units for the dietary exposure to "0.45 mg TOS/kg bw/day" from "0.45 TOS/kg bw/day" and correct the notifier name in the Standards of Identity paragraph.

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001201. We received Chr. Hansen A/S (Chr. Hansen)'s notice on June 18, 2024 and filed it on September 10, 2024. Chr. Hansen submitted amendments to the notice on January 17, 2025 and March 5, 2025, containing additional information on enzyme identity, the enzyme safety narrative, and specifications.

The subject of the notice is lipase enzyme preparation produced by *Komagataella phaffii* expressing a gene encoding lipase from *Yarrowia lipolytica* (lipase enzyme preparation) for use as an enzyme at up to 95.1 mg Total Organic Solids (TOS)/kg raw material in the production of cheese, enzyme-modified dairy ingredients, and plant-based analogues of milk and milk products. The notice informs us of Chr. Hansen's view that this use of lipase 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 lipase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, lipase is identified by the Enzyme Commission Number 3.1.1.3,[1] and is identified by the Chemical Abstracts Service as CAS

[1] https://iubmb.qmul.ac.uk/enzyme/EC3/1/1/3.html

Number 9001-62-1. Chr. Hansen states that the primary amino acid sequence of the lipase consists of 349 amino acids with a molecular weight of 38.8kDa.

Chr. Hansen states that the *K. phaffii* production organism is a non-pathogenic and non-toxigenic yeast with a history of safe use in food production.
Chr. Hansen states that the *K. phaffii* production strain "DSM 34125" was constructed from the host strain by targeted integration of an expression cassette carrying a lipase gene from *Y. lipolytica*. 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 antibiotic resistance genes as confirmed by whole genome sequencing.

Chr. Hansen states that lipase enzyme preparation is manufactured by controlled fermentation of a pure culture of the *K. phaffii* 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 steps. The lipase enzyme concentrate is formulated to a liquid enzyme preparation with other ingredients, including glycerol, sorbitol, and water, resulting in a colorless to amber liquid. 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 lipase 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 lipase 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 lipase 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 lipase enzyme preparation at a maximum level of 95.1 mg TOS/kg raw material in the production of cheese, enzyme-modified dairy ingredients, and plant-based analogues of milk and milk products to catalyze the hydrolysis of tri-, di-, and mono-glycerides to yield free fatty acids, monoglycerides, diglycerides, and glycerol. Chr. Hansen notes that the lipase enzyme is removed, denatured, and/or inactivated during food production. Chr. Hansen estimates a maximum dietary exposure to the lipase enzyme preparation to be 0.45 mg TOS/kg bw/day from the use in food and drinks with the assumption that the added lipase enzyme preparation remains present in the final food.[2]

Chr. Hansen relies on published information that discusses the safety of the *K. phaffii*

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

production organism, the safety of microbial enzyme preparations used in food processing, the safety of the *Y. lipolytica* donor organism, and the safety of lipase enzymes. Chr. Hansen summarizes the available published literature that supports the history of safe use of lipases in food. Chr. Hansen states that the notified lipase amino acid sequence is the exact same as that which is natively produced in *Y. lipolytica*, and humans have safely consumed lipase from *Y. lipolytica* as this yeast is common in a variety of foods. Furthermore, Chr. Hansen notes that no treatment-related adverse effects have been observed in toxicological studies using lipase enzymes produced by different production organisms. Chr. Hansen concludes that these studies serve as corroborative evidence to bridge to the safety of the notified lipase enzyme preparation given that enzymes within the same activity class share highly conserved active site regions, and changes in other regions of the enzyme are not expected to impact safety of the lipase. Chr. Hansen summarizes the toxicological studies performed using enzymes produced by related *K. phaffii* production organisms to support its safety as a production organism for lipase enzyme preparation. In addition, Chr. Hansen states that enzymes are generally added at the lowest level to catalyze the desired reaction and that exposure is generally low. Also, Chr. Hansen states that a literature search did not identify any information that would contradict a general recognition of safety of the lipase 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 lipase. 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 *Y. lipolytica* lipase 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 lipase will result in allergenic responses from its intended uses.

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

Standards of Identity

In the notice, Chr. Hansen states its intention to use lipase 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

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 lipase 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 lipase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing lipase 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 lipase enzyme preparation is GRAS under its intended conditions of use. This letter is not an affirmation that lipase 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 001201 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2025.07.03 11:17:43 -04'00'

Susan Carlson, Ph.D.
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