



Dr. Kritika Mahadevan
Clara Foods Co.
1 Tower Place, Suite 800
South San Francisco, CA 94080

Re: GRAS Notice No. GRN 001025

Dear Dr. Mahadevan:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001025. We received Clara Foods Co.'s (Clara) notice on August 2, 2021 and filed it on November 5, 2021.¹ Clara submitted amendments to the notice on November 15, 2021 and October 5, 2022 that provided updated contact information and clarifications on the identity, method of manufacturing, specifications, dietary exposure, and the safety narrative.

The subject of the notice is pepsin A enzyme preparation produced by *Komagataella phaffii* strain DFB-002² expressing a gene encoding the proenzyme pepsinogen A from *Sus scrofa*. The proenzyme is subsequently converted (by pH and temperature adjustment) to pepsin A (pepsin A enzyme preparation) for use as an enzyme at a maximum level of 14 mg total organic solids (TOS)/kg milk in the production of cheese, 10 mg TOS/kg raw material for vegetable protein hydrolysis, and up to 34 mg TOS/kg raw material in the production of beer.³ The notice informs us of Clara's view that this use of pepsin A 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. Clara's notice provides information about the components in the pepsin A enzyme preparation.

¹ The notice was submitted by Kevin O. Gillies, consultant, on behalf of Clara Foods, as reflected in the filing letter issued by FDA. In an amendment received November 15, 2021, we were informed that the new contact person for the notice was Dr. Kritika Mahadevan of Clara Foods.

² Clara refers to the production organism as *Pichia pastoris* DFB-002 in GRN 001025, but also notes that *P. pastoris* has been reclassified as *Komagataella phaffii* as described by Kurtzman, C. (2005). Description of *Komagataella phaffii* sp. nov. and the transfer of *Pichia pseudopastoris* to the methylotrophic yeast genus *Komagataella*. International Journal of Systematic and Evolutionary Microbiology, 55, 973-976. doi: 10.1099/ijs.0.63491-0.

³ Clara Foods states that the intended uses of pepsin A enzyme preparation are substitutional to the uses of porcine pepsin A (CAS Reg. No. 9001-75-6) as defined in 21 CFR 184.1595.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, pepsin A is identified by the Enzyme Commission Number 3.4.23.1.⁴ Clara states that the primary sequence of pepsin A is 326 amino acids with a molecular weight of 34.5 kDa.

Clara discusses published literature on *K. phaffii* and the characterization of the pepsin A enzyme production strain and confirms that the *K. phaffii* yeast production organism is non-pathogenic and non-toxicogenic. Clara states that the recipient strain^{5,6} used in the construction of the production strain, “DFB-002,” was genetically engineered from the commercially-available base strain, *K. phaffii* strain “BG10.” Clara states that the production organism was constructed through transformation with an expression cassette carrying the porcine Pepsinogen A gene, copies of *K. phaffii* methanol-inducible promoters, and *Saccharomyces cerevisiae* alpha mating factor pre-pro sequence for the expression of heterologous proteins in *K. phaffii*.⁷ Clara states that it confirmed sequence integration by whole genome sequencing. Clara evaluated the genetic stability of the production strain by monitoring the presence of integrated genes after 45 generations of growth on non-selective growth media. Clara states that no plasmids are present in the production strain. Clara also verified the absence of functional antibiotic resistance genes by polymerase chain reaction and genome sequencing. Clara concludes that the genome of production strain DFB-002 is fully sequenced and well-characterized.

Clara states that the pepsin A enzyme preparation is manufactured by fermentation of a pure culture of the *K. phaffii* DFB-002 production strain under controlled conditions. Pepsinogen A enzyme is secreted into the fermentation medium, converted to pepsin A through pH and temperature adjustments, and then recovered by centrifugation and microfiltration. This is followed by additional pH adjustment, ultrafiltration, and drying. Clara states that the fermentation medium does not contain any major allergens. Clara states that the entire process is performed using food grade raw materials and in accordance with current good manufacturing practices.

Clara has established food grade specifications and states that the pepsin A enzyme preparation conforms to the general specifications for enzymes in the Food Chemicals Codex (FCC, 10th ed., 2016)⁸ 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). Clara provides results from analyses of three non-consecutive batches of pepsin A enzyme preparation to

⁴ <https://iubmb.qmul.ac.uk/enzyme/EC3/4/23/1.html>

⁵ Clara states that the parental strain, “BGO8,” is an isolate from the Phillips Petroleum strain NRRL Y-11430 obtained from the Agriculture Research Service culture collection (Peoria, IL, USA).

⁶ Clara states that the recipient *K. phaffii* strain was obtained by selecting for the absence of cytoplasmic killer plasmids in the parental strain and modification to develop a methanol-utilization slow phenotype.

⁷ Clara states that the production strain is deposited with the American Type Culture Collection under the accession number GSD-1197.

⁸ Specifications for enzymes remain the same in the most recent edition of the Food Chemicals Codex (FCC, 13th edition, 2022).

demonstrate that the manufacturing acceptance criteria have been met, including the absence of both the production organism and antibiotic activity.

Clara intends to use pepsin A enzyme preparation to catalyze the hydrolysis of peptide bonds between hydrophobic or aromatic residues of protein. Clara notes that the pepsin A enzyme preparation is intended as a direct replacement for existing uses of porcine pepsin A as an enzyme. Clara discusses the retention of the enzyme in foods as consumed, noting that much of the enzyme is lost in the whey fraction during cheesemaking and in filtration steps of beermaking, but that enzyme added for plant protein hydrolysis is likely retained in the final hydrolyzed protein. Clara bases its estimates of exposure on the use of the enzyme at 10 g TOS/kg plant protein. Using published estimates of usual protein intake for ages 1+ years (U.S. Department of Agriculture, 2021) based on 2015-2018 NHANES data,⁹ and the assumption that one-third of all protein consumed in the U.S. is plant protein containing the enzyme at levels of 10 g TOS/kg protein,¹⁰ Clara estimates the mean and 90th percentile dietary exposure to pepsin A enzyme preparation to be 5 and 7 mg TOS/kg body weight per day (mg TOS/kg bw/d), respectively, from the intended uses.

Clara relies on published information that discusses the safety of the *K. phaffii* production organism, including safe strain lineage and use of the parent strain for production of food ingredients, and the safety of microbial enzyme preparations used in food processing. Clara states that pepsin A enzyme is identical to native porcine pepsin A at the amino acid level, and, based on molecular weight, immunoreactivity, and enzyme activity, pepsin A enzyme preparation is similar to porcine pepsin A enzyme as defined in 21 CFR 184.1595. In support of the safety of pepsin A enzyme preparation, Clara summarizes the history of safe use of porcine pepsin A in food.

Clara states that there is no evidence of allergenicity risk related to the consumption of pepsin A enzyme preparation. Additionally, Clara discusses a published study suggesting that there is no evidence of allergenicity from the fermentation products of *K. phaffii*.

Based on the data and information summarized above, Clara concludes the pepsin A enzyme preparation is GRAS for its intended use.

Standards of Identity

In the notice, Clara states its intention to use pepsin A enzyme preparation in several food categories, including foods for which standards of identity exist, located in Title 21

⁹ USDA, Agricultural Research Service, 2021. Usual Nutrient Intake from Food and Beverages, by Gender and Age, What We Eat in America, NHANES 2015-2018. Available: www.ars.usda.gov.

¹⁰ Hoy MK, Clemens JC, Moshfegh AJ. Protein intake of adults in the U.S.: What We Eat in America, NHANES 2015-2016. Food Surveys Research Group Dietary Data Brief No. 29. January 2021. Available at: <https://www.ars.usda.gov>.

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 Clara's notice concluding that pepsin A 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 pepsin A enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing pepsin A enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Clara provided, as well as other information available to FDA, we have no questions at this time regarding Clara's conclusion that pepsin A enzyme preparation produced by *K. phaffii* is GRAS under its intended conditions of use. This letter is not an affirmation that pepsin A enzyme preparation produced by *K. phaffii* 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 001025 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J.
Carlson -S

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Susan Carlson, Ph.D.
Director
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