U.S. FOOD & DRUG ADMINISTRATION
Human Foods Program

Charles Denby, Ph.D.
Berkeley Fermentation Science Inc.
15555 E 14th St, Ste 525
San Leandro, CA 94578

Re: GRAS Notice No. GRN 001246

Dear Dr. Denby:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001246. We received Berkeley Fermentation Science Inc. (Berkeley)'s notice on October 23, 2024, and filed it on April 9, 2025. Berkeley submitted amendments to the notice on August 15, 2025, and September 3, 2025, containing additional information regarding the production organism, intended use, manufacturing process, analytical methods, specifications, and safety narrative.

The subject of the notice is *Saccharomyces cerevisiae* "BY-1248" for use at a level of 10^7 cells/mL of grape must in wine fermentation to enhance the flavor profile of the finished wine. The notice informs us of Berkeley's view that this use of *S. cerevisiae* "BY-1248" is GRAS through scientific procedures.

Berkeley describes the ingredient as a beige to light brown liquid slurry. Berkeley discusses the identity and construction of *S. cerevisiae* "BY-1248" and states that *S. cerevisiae* "BY-1248" is non-pathogenic and non-toxigenic. Berkeley states that *S. cerevisiae* "BY-1248" was constructed from an industrial winemaking strain of *S. cerevisiae* that has been used in commercial wine production for decades. *S. cerevisiae* "BY-1248" was constructed using Golden Gate Assembly to insert the alcohol-O-acyltransferase (AAT) and fatty acid synthase (FAS) gene sequences, a promoter DNA sequence, and a terminator DNA sequence. Berkeley states that the promoter and terminator sequences aid in gene transcription and translation, while the AAT sequence encodes an AAT enzyme, and the FAS sequence encodes a FAS enzyme. Berkeley states that the AAT and FAS enzymes aid in the production of ethyl hexanoate and hexanoic acid, respectively, which are compounds that contribute fruit flavor and aroma to alcoholic beverages. Berkeley discusses the results of genotypic and phenotypic analyses and concludes that *S. cerevisiae* "BY-1248" contains the insertion of the AAT, FAS, promoter, and terminator sequences but does not contain the antibiotic resistance gene used for selection during strain development.

Berkeley discusses the manufacturing process for *S. cerevisiae* "BY-1248," stating that the fermentation process is performed in a sterile, contained environment under controlled conditions. After fermentation is complete, Berkeley states that the temperature of the culture is lowered, and the cells are separated through natural

processes of flocculation and settling. Berkeley notes that the yeast cell mass is collected as a concentrated liquid slurry and stored at 4 °C until used to inoculate the wine fermentation. Berkeley states that *S. cerevisiae* "BY-1248" is manufactured in accordance with current good manufacturing practices and that all raw materials and processing aids are food grade and are used in accordance with U.S. regulations, are the subject of an effective food contact notification, or are concluded to be GRAS for their intended use. Berkeley further notes that none of the components used in the manufacturing process are major allergens or are derived from major allergens.

Berkeley provides specifications for *S. cerevisiae* "BY-1248" that include viable yeast cells (> 95%), and limits for lead (< 10 µg/kg), total bacteria (< 1 per 1×10^6 yeast cells), total wild yeast (< 5 per 1×10^6 yeast cells), and microorganisms, including *Escherichia coli* (0 per 1×10^6 yeast cells). Berkeley provides the results from the analyses of five non-consecutive batches to demonstrate that *S. cerevisiae* "BY-1248" can be manufactured to meet the specifications.

Berkeley states that *S. cerevisiae* "BY-1248" is removed from wine as part of the standard winemaking process and the finished wine will contain trace levels of the yeast. Therefore, the dietary exposure to *S. cerevisiae* "BY-1248" is negligible. Berkeley states that the intended use of *S. cerevisiae* "BY-1248" is substitutional for the use of other *S. cerevisiae* strains currently used in commercial wine brewing and therefore, the dietary exposure to *S. cerevisiae* is not expected to increase.

Berkeley reports the levels of ethyl hexanoate (6.4-8.7 mg/L) and hexanoic acid (19.7-24.1 mg/L) present in wine produced using *S. cerevisiae* "BY- 1248". Berkeley presumed that all wine is produced with *S. cerevisiae* "BY-1248" and estimates the 90th percentile dietary exposure to ethyl hexanoate and hexanoic acid to be 11 mg/person (p)/day (d) and 30.5 mg/p/d, respectively, using the highest levels of ethyl hexanoate and hexanoic acid found in the wine and wine consumption data from the 2005-2014 National Health and Nutrition Examination Survey.

Berkeley explains the safety of *S. cerevisiae* "BY-1248" by discussing the safety of the *S. cerevisiae* species, donor strains, and the FAS and AAT enzymes. Berkeley notes that *S. cerevisiae* has a safe history of use in food and does not produce toxins. Berkeley states that *S. cerevisiae* has safely been used in fermentation processes and that the parent strain has been extensively used in commercial wine production. Berkely further notes that the gene sequences for the FAS enzyme, promoter, and terminator sequences are derived from *S. cerevisiae* species. Berkeley discusses the gene sequence for the AAT enzyme, stating that it was *de novo* synthesized and there is no possibility of transfer of genetic material from the donor strain, *Marinobacter aquaeolei*. Berkeley states that wine fermented with *S. cerevisiae* "BY-1248" contains ethyl hexanoate and hexanoic acid produced by the AAT and FAS enzymes, respectively, but notes that the concentrations of these compounds are expected to be similar to or lower than what is present in other foods and beverages.

Based on the totality of the data and information, Berkeley concludes that *S. cerevisiae* "BY-1248" is GRAS for its intended use.

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 Berkeley's notice concluding that *S. cerevisiae* "BY-1248" 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 *S. cerevisiae* "BY-1248." Accordingly, our response should not be construed to be a statement that foods containing *S. cerevisiae* "BY-1248," if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Berkeley provided, as well as other information available to FDA, we have no questions at this time regarding Berkeley's conclusion that *S. cerevisiae* "BY-1248" is GRAS under its intended conditions of use. This letter is not an affirmation that *S. cerevisiae* "BY-1248" 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 001246 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2025.09.22 10:24:45 -04'00'

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Director
Division of Food Ingredients
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