U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

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

Re: GRAS Notice No. GRN 001265

Dear Dr. Denby:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001265. We received Berkeley Fermentation Science Inc. (BFS)'s notice on March 26, 2025, and filed it on July 10, 2025. BFS submitted an amendment to the notice on October 1, 2025, that provided additional information about the manufacturing process, specifications, analytical methods, and dietary exposure.

The subject of the notice is *Saccharomyces cerevisiae* "BY-1532" with deletions of four genes involved in maltose and maltotriose metabolism and carrying a gene encoding an alcohol-O-acyltransferase (AAT) enzyme from *S. cerevisiae* and a gene encoding a carbon-sulfur lyase (CSL) from *Citrobacter freundii* (*S. cerevisiae* "BY-1532"), for use at a level of 7.5×10^6 cells/mL wort in the production of non-alcoholic beer (<0.5% alcohol by volume (ABV)) to enhance the flavor profile. The notice informs us of BFS' view that this use of *S. cerevisiae* "BY-1532" is GRAS through scientific procedures.

BFS describes *S. cerevisiae* "BY-1532" as a liquid slurry of the strain. During non-alcoholic beer fermentation using the strain, reduced levels of the sugars maltose and maltotriose are consumed and the AAT and CSL enzymes are expressed. Reduced consumption of maltose and maltotriose results in beer with less than 0.5% ABV. Expression of the AAT enzyme results in the release of acetate esters (ethyl acetate, isoamyl acetate, and 2-phenylethyl acetate) imparting a fruity/beer-like aroma. Expression of the CSL enzyme results in the release of the volatile thiol compounds 3-mercapto-1-hexanol (3MH) and 3-mercaptohexyl acetate (3MHA), imparting a tropical fruit flavor and aroma to the beer. BFS states that *S. cerevisiae* "BY-1532" was constructed from an *S. cerevisiae* strain with extensive use in commercial beer production. The parent strain was modified by: 1. Deletions of three genes involved in maltose and maltotriose metabolism, encoding a transporter, a maltase, and a transcription factor; 2. Deletion of a gene encoding a maltose-specific transporter; 3. Targeted integration of an expression cassette carrying a gene from *S. cerevisiae* encoding an AAT enzyme under the control of a promoter and terminator from *S. cerevisiae*, and 4. Targeted integration of an expression cassette carrying a gene from *C. freundii* encoding a CSL enzyme under the control of a promoter and terminator from *S. cerevisiae*. BFS states that the sequence integrity of the expression cassettes was

confirmed by DNA sequencing, and the deletions and integrations at the target loci and genetic stability were confirmed by polymerase chain reaction (PCR). BFS states that *S. cerevisiae* "BY-1532" is non-pathogenic and non-toxigenic and does not contain any antibiotic resistance genes. BFS notes that genetically modified *S. cerevisiae* strains have been the subjects of previous GRNs.[1,2]

BFS describes the method of manufacture of *S. cerevisiae* "BY-1532" as fermentation of a pure culture of *S. cerevisiae* "BY-1532" under controlled conditions. After fermentation, the culture is cooled, the yeast cells are separated from the fermentation medium by flocculation and settling, and the yeast cell slurry is collected. BFS states that none of the components of the manufacturing process include or are derived from major food allergens. BFS states that *S. cerevisiae* "BY-1532" is manufactured in accordance with current good manufacturing practices and that all materials used in the manufacturing process are food-grade. BFS further states that all materials used in the manufacturing process are used in accordance with applicable U.S. regulations, are the subject of an effective food contact notification, or are concluded to be GRAS for the intended use.

BFS provides specifications for *S. cerevisiae* "BY-1532" that include viable cell count (> 95%) and limits for lead (< 0.01 mg/kg) and microorganisms, including total bacteria (absent in 1×10^6 yeast cells) and total wild yeast (absent in 1×10^6 yeast cells). BFS provides the results from the analyses of four non-consecutive batches to demonstrate that *S. cerevisiae* "BY-1532" can be manufactured to meet the specifications.

BFS states that the intended use of *S. cerevisiae* "BY-1532" is substitutional for the use of other *S. cerevisiae* strains currently used in the brewing of non-alcoholic beer and therefore, the dietary exposure to *S. cerevisiae* is not expected to increase. BFS states that *S. cerevisiae* "BY-1532" is removed from the beer as part of the standard brewing process and therefore, the finished beer will contain only trace levels of the yeast and the dietary exposure to *S. cerevisiae* BY-1532 is negligible. BFS reports that levels of the flavoring compounds (ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, 3MH, and 3MHA) present in beer produced using *S. cerevisiae* "BY-1532" are similar to or lower than the levels in other commercial beers and therefore, the dietary exposure to these flavoring compounds from the intended uses of *S. cerevisiae* "BY-1532" is substitutional.

BFS discusses publicly available data and information supporting the safety of the enzymes (AAT and CSL), the acetate esters (ethyl acetate, isoamyl acetate, and 2-phenylethyl acetate), and the volatile thiols (3MH and 3MA). BFS characterizes AAT as an enzyme that transfers acyl chains from an acyl-CoA donor to an acceptor alcohol, resulting in the production of an acyl ester. BFS characterizes CSL as an enzyme that

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[1] *S. cerevisiae* strains are the subjects of GRNs 000120, 000175, 000350, 000798, 000841, 001062, 001094, and 001096. We evaluated these notices and responded in letters dated June 30, 2003, January 6, 2006, February 4, 2011, August 13, 2019, March 10, 2020, April 5, 2023, November 28, 2023, and July 5, 2023, stating that we had no questions at that time about the notifiers' GRAS conclusions.

[2] BFS states that the CSL enzyme in *S. cerevisiae* "BY-1532" is a re-application of the genetic engineering detailed in GRN 001094.

cleaves the carbon-sulfur bond in glutathione-3MH and cysteine-3MHA. Based on the results of *in silico* sequence alignment-based approaches, BFS concludes that neither the AAT enzyme nor the CSL enzyme pose an allergenic or toxigenic risk to consumers. BFS notes that dietary exposures to the AAT and CSL enzymes will likely be negligible due to cytoplasmic expression of the enzymes and the removal of *S. cerevisiae* "BY-1532" during beer production.

BFS states that ethyl acetate is a synthetic flavoring substance and adjuvant that is listed as GRAS under 21 Code of Federal Regulations (CFR) 182.60 and may be safely used in food as a secondary direct food additive permitted in food for human consumption under 21 CFR 173.228. BFS states that isoamyl acetate and 2-phenylethyl acetate are approved synthetic flavoring substances permitted for direct addition to food under 21 CFR 172.515. Additionally, BFS references the independent reviews of the safety of ethyl acetate, isoamyl acetate, and 2-phenylethyl acetate by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). BFS notes that no safety concerns regarding the use of ethyl acetate, isoamyl acetate, or 2-phenylethyl acetate were identified by the JECFA.

BFS discusses that 3MH and 3MHA are well-known flavor molecules that have commonly been consumed in food. BFS references the independent review on the safety of simple aliphatic and aromatic sulfides and thiols by the JECFA Committee. BFS notes that no safety concerns regarding the use of 3MH and 3MHA as flavoring agents were identified by the JECFA based on current intake levels. Additionally, BFS notes no unexpected changes in the metabolite composition related to beer fermentation utilizing *S. cerevisiae* "BY-1532".

BFS conducted a comprehensive literature search through March 2025 to identify available safety information relevant to the AAT enzyme, the generated acetate esters ethyl acetate, isoamyl acetate, and 2-phenylethyl acetate, the CSL enzyme, and the generated volatile thiols 3MH and 3MHA. BFS did not identify any safety concerns or information that would contradict its GRAS conclusion.

Based on the totality of data and information, BFS concludes that *S. cerevisiae* "BY-1532" is GRAS under the conditions of 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 BFS's notice concluding that *S. cerevisiae* "BY-1532" 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-1532". Accordingly, our response should not be construed to be a statement that foods containing *S. cerevisiae* "BY-1532", if introduced or delivered for introduction into

interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that BFS provided, as well as other information available to FDA, we have no questions at this time regarding BFS's conclusion that *S. cerevisiae* "BY-1532" is GRAS under its intended conditions of use. This letter is not an affirmation that *S. cerevisiae* "BY-1532" 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 001265 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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Date: 2025.12.11 12:10:25 -05'00'

Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Pre-Market Additive Safety
Office of Food Chemical Safety, Dietary Supplements, and Innovation
Human Foods Program