Charles Denby, Ph.D.
Berkeley Fermentation Science Inc.
2451 Peralta Street
Oakland, CA 94607

Re: GRAS Notice No. GRN 001094

Dear Dr. Denby:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001094. We received Berkeley Fermentation Science Inc.'s (BFS) notice on June 8, 2022 and filed it on February 7, 2023. BFS submitted amendments to the notice on June 9, 2023 and September 15, 2023 that provided additional information about the manufacturing process, specifications, analytical methods, and information on the dietary exposure and safety of flavor molecules produced during beer fermentation.

The subject of the notice is *Saccharomyces cerevisiae* strain "BY-989" carrying a gene encoding a carbon-sulfur lyase (CSL) from *Citrobacter freundii* (*S. cerevisiae* "BY-989") for use at up to 1.5 x 10^7 cells/mL wort in the production of beer to enhance the flavor profile. The notice informs us of BFS' view that this use of *S. cerevisiae* "BY-989" is GRAS through scientific procedures.

BFS describes *S. cerevisiae* "BY-989" as a liquid slurry of the strain. During beer fermentation using the strain, the CSL enzyme is expressed and the volatile thiol compounds, 3-mercapto-1-hexanol (3MH) and 3-mercaptohexyl acetate (3MHA), are released and impart a tropical fruit flavor and aroma to the beer. BFS states that *S. cerevisiae* "BY-989" was constructed from *S. cerevisiae* strain London Ale Yeast (LAY), a brewer's yeast strain commonly used in commercial beer production, by targeted integration of an expression cassette carrying a modified gene[1] from *C. freundii* encoding a CSL under the control of a promoter and a terminator from *S. cerevisiae*. BFS states that the sequence integrity of the expression cassette was confirmed by DNA sequencing, the integration at the target locus and genetic stability by PCR. BFS states that *S. cerevisiae* "BY-989 is non-pathogenic and non-toxigenic and does not contain any antibiotic resistance genes.

BFS describes the method of manufacture of *S. cerevisiae* "BY-989"as a controlled fermentation of a pure culture of *S. cerevisiae* "BY-989." After fermentation, the yeast cell slurry is collected and diluted to a target density and packaged. BFS states that none of the components of the manufacturing process include or are derived from major

[1] The CSL gene was codon optimized and encodes CSL with five amino acid changes to increase specificity to conjugated thiols.

allergens. BFS states that *S. cerevisiae* "BY-989" is produced in accordance with current good manufacturing practices and that all materials used in the manufacturing process are food-grade and are used in accordance with applicable U.S. regulations.

BFS provides specifications for *S. cerevisiae* "BY-989" that include viable cell count (>95%) and limits for bacteria and yeast (0 CFU per 2.5 x 10^8 yeast cells) and lead (0.01 mg/kg). BFS provides the results from the analyses of three non-consecutive batches to demonstrate that the ingredient can be manufactured to meet the specifications.

BFS states that the intended use of *S. cerevisiae* "BY-989" is substitutional for the use of other *S. cerevisiae* strains currently used in commercial beer brewing and therefore, the dietary exposure to *S. cerevisiae* is not expected to increase. BFS states that *S. cerevisiae* "BY-989" is removed from beer as part of the standard brewing process and the finished beer will contain trace levels of the yeast. BFS reports the levels of volatile thiols, 3MH (7-9 µg/L) and 3MHA (0.3-1 µg/L), present in beer produced using *S. cerevisiae* "BY-989." BSF estimates the dietary exposure to 3MH and 3MHA for high consumers of beer to be 12.78 µg/person (p)/d and 1.42 µg/p/d, respectively, for males, and 9.6 µg/person (p)/d and 1.07 µg/p/d, respectively, for females. BFS indicates levels of 3MH and 3MHA present in beer produced using *S. cerevisiae* "BY-989" are substitutional for levels already present in beer fermented with highly aromatic hop preparations and the intended use of *S. cerevisiae* "BY-989" will not increase cumulative dietary exposure to 3MH and 3MHA.

BFS discusses publicly available data and information supporting the safety of the CSL enzyme expressed by *S. cerevisiae* "BY-989", and generated volatile thiols aroma/flavor compounds, 3MH and 3MHA. BFS characterizes CSL as a carbon-sulfur lyase enzyme that cleaves the carbon-sulfur bond in glutathione-3MH and cysteine-3MH. BFS discusses expression of CSL enzymes by endogenous microbiota, indicating this activity is already common in the human gastrointestinal tract. Based on the results of *in silico* sequence alignment-based approaches, BFS concludes that the CSL does not pose an allergenic or toxigenic risk to consumers. BFS notes that dietary exposure to CSL will likely be negligible due to cytoplasmic expression of CSL and removal during the process of beer production.

BFS states 3MH and 3MHA are well-known flavor molecules with a tropical fruit sensory profile and previous widespread human consumption as a component in food. BFS references the independent review on the safety of simple aliphatic and aromatic sulfides and thiols by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). BFS notes that no safety concerns regarding the use of 3MH and 3MHA as flavoring agents were identified by the JECFA expert panel based on current intake levels. BFS notes no unexpected changes in the metabolite composition related to beer fermentation utilizing *S. cerevisiae* "BY-989."

BFS conducted a comprehensive literature search through June 2023 to identify available safety information relevant to the CSL enzyme expressed by *S. cerevisiae* "BY-989" and generated volatile thiols aroma/flavor compounds 3MH and 3MHA. BFS did not identify any safety concerns or information that would contradict its GRAS

conclusion.

Based on the totality of information, BFS concludes that *S. cerevisiae* "BY-989" 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-989" 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-989." Accordingly, our response should not be construed to be a statement that foods containing *S. cerevisiae* "BY-989," 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-989" is GRAS under its intended conditions of use. This letter is not an affirmation that *S. cerevisiae* "BY-989" 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 0001094 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.11.28 18:20:20 -05'00'

Susan Carlson, Ph.D.
Director
Division of Food Ingredients
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and Applied Nutrition