U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

Mary M. Murphy, MS, RD
Exponent
1150 Connecticut Ave., NW
Washington, DC 20036

Re: GRAS Notice No. GRN 001142

Dear Ms. Murphy:

The Food and Drug Administration (FDA, we) completed our evaluation of the supplement that you submitted on behalf of Oobli, Inc. (Oobli) to GRN 001142. We received the supplement on August 5, 2024. The supplement addresses changes in the method of manufacture, production organism, and specifications for the subject of GRN 001142. Oobli submitted an amendment on May 9, 2025, clarifying the specifications.

We previously responded to GRN 001142 on March 11, 2024. We stated that we had no questions at that time regarding Oobli's conclusion that brazzein preparation produced by *Komagataella phaffii* P-BRZ-013[1] expressing the gene encoding for the minor isoform (53 amino acid) of brazzein from *Pentadiplandra brazzeana* (brazzein preparation) is GRAS for its intended use as a general-purpose sweetener in food at levels determined by current good manufacturing practices.

In the supplement dated August 5, 2024, Oobli informs us of its view that brazzein preparation produced by *K. phaffii* P-BRZ-336 is GRAS, through scientific procedures, for the same uses described in GRN 001142. GRN 001142 excludes use in infant formula and foods under the jurisdiction of the U.S. Department of Agriculture. Oobli states that the genetically engineered, non-pathogenic, and non-toxigenic *K. phaffii* P-BRZ-013 may be replaced with the genetically engineered, non-pathogenic, and non-toxigenic *K. phaffii* P-BRZ-336 to produce the major isoform (54 amino acid) of brazzein. In the supplement, Oobli also informs us of changes in the method of manufacture used to produce both isoforms of brazzein.

Our use of the term, "brazzein preparation," in this letter is not our recommendation of that term as an appropriate common or usual name for declaring the substance in accordance with FDA's labeling requirements. Under 21 CFR 101.4, each ingredient must be declared by its common or usual name. In addition, 21 CFR 102.5 outlines general principles to use when establishing common or usual names for

[1] FDA notes that *Pichia pastoris* was reclassified as *K. phaffii* as reported in Kurtzman, 2005 (Ref. 1).

nonstandardized foods. Issues associated with labeling and the common or usual name of a food ingredient are under the purview of the Office of Nutrition and Food Labeling (ONFL) in the Nutrition Center of Excellence. The Office of Pre-Market Additive Safety (OPMAS) did not consult with ONFL regarding the appropriate common or usual name for "brazzein preparation."

Oobli notes that brazzein naturally present in the fruit of *P. brazzeana* Baillon occurs as both the major (54 amino acid) and minor (53 amino acid) isoforms. The minor isoform lacks the N-terminal pyroglutamic acid residue of the major isoform. In the supplement, Oobli states that it intends to use brazzein preparation, containing either (or a combination) of the major or minor isoforms of brazzein, as a general-purpose sweetener in food at levels determined by good manufacturing practices.

In GRN 001142, Oobli stated that brazzein preparation contains primarily protein (approximately 80%), of which approximately 40% of the total protein is the minor isoform of brazzein, and the remaining total protein consists of *K. phaffii* proteins carried over from the fermentation process. The ingredient also contains carbohydrates, ash, moisture, and fat. In the supplement, Oobli states that brazzein preparation contains primarily protein (≥ 65%), of which ≥ 40% is the major and/or minor isoform of brazzein. Oobli provides the results of a proteomics assessment of brazzein preparation to determine the identities of the *K. phaffii* proteins present in the final ingredient.

In the supplement, Oobli provides information on an alternative production organism, *K. phaffii* P-BRZ-336, genetically engineered to express the major isoform of brazzein. Oobli states that the organism is a well-characterized yeast with a history of safe use in the food industry. Oobli states that the production organism is derived from *K. phaffii* BG10, which is derived from *K. phaffii* NRRL Y-11430. Oobli states that the production organism is constructed through a series of transformations with different expression constructs to enable the production of brazzein. Oobli states that the production organism does not contain any antibiotic resistance genes or plasmids and is not capable of transferring plasmids or antibiotic resistance to other organisms.

Oobli states that brazzein preparation is manufactured using a method similar to the one described in GRN 001142; however, in the supplement, the chromatography step is omitted. Oobli states that the revised method of manufacture is used to produce both isoforms of brazzein. The manufacturing process starts with the controlled fermentation of the production organism. Oobli states that none of the raw materials used during the manufacturing process are allergens or are derived from major allergens. Brazzein is secreted into the fermentation medium and then separated from the biomass. The fermentation supernatant containing brazzein is concentrated, pH-adjusted, filtered, and then spray-dried to obtain the final powder form of brazzein. Oobli states that brazzein preparation is manufactured in accordance with current good manufacturing practices.

Oobli provides updated specifications for brazzein preparation that include total protein content (≥65% w/w), brazzein as percent of total mass (≥40% w/w), and limits for

moisture (≤10% w/w), ash (≤10% w/w), fat (≤1% w/w), carbohydrates (≤30% w/w), heavy metals, including lead (≤0.1 mg/kg), and microorganisms, including *Escherichia coli* (<10 colony forming unit/g), *Salmonella* spp. (not detected in 25 g) and *Listeria* (not detected in 25 g). Oobli provides the results from the analyses of three non-consecutive batches of brazzein preparation containing the major isoform and three non-consecutive batches of brazzein preparation containing the minor isoform to demonstrate that the ingredients can be manufactured to meet the stated specifications. Oobli also discusses the results of a shelf-life stability study of brazzein preparation containing the major isoform of brazzein. Brazzein preparation was stored under ambient storage conditions (23 °C) for 12 months. Oobli reports that the results of the study demonstrate that brazzein preparation remained stable over the storage period.

In GRN 001142, Oobli provided estimates of dietary exposure to brazzein preparation based on its relative sweetness intensity and the methodology presented in Renwick, 2008 (Ref. 2). This study reported average and upper percentile (i.e., 90th percentile and higher) estimates of dietary exposure to intense sweeteners among children and adults with and without diabetes and estimated the dietary exposure to a sweetener based on its relative sweetness and an assumption of its substitutional use. In the supplement, Oobli reports that the major isoform of brazzein has a sweetness intensity that is approximately 550 times that of sucrose and estimates that brazzein preparation, containing an average of 51% of the major isoform of brazzein, is approximately 281 times sweeter than sucrose. Similarly, Oobli reports that the minor isoform of brazzein has a sweetness intensity that is approximately 750 times that of sucrose and estimates that brazzein preparation, containing an average of 48% of the minor isoform of brazzein, is approximately 360 times sweeter than sucrose. Oobli estimates the average and upper percentile dietary exposures to brazzein preparation containing the major isoform of brazzein for non-diabetic adults (0.91 and 2.41 mg/kg body weight (bw)/d); diabetic adults (1.00 and 3.20 mg/kg bw/d); non-diabetic children (1.52 and 3.53 mg/kg bw/d); and diabetic children (2.40 and 3.24 mg/kg bw/d), respectively. Oobli estimates the average and upper percentile dietary exposures to brazzein preparation containing the minor isoform of brazzein for non-diabetic adults (0.71 and 1.88 mg/kg bw/d); diabetic adults (0.78 and 2.49 mg/kg bw/d); non-diabetic children (1.18 and 2.75 mg/kg bw/d); and diabetic children (1.87 and 2.52 mg/kg bw/d), respectively.

Oobli conducted an updated review of the scientific literature through May 2025 and states that it did not identify any new information inconsistent with their conclusion that brazzein preparation containing the major and/or minor isoform of brazzein is GRAS under the conditions of its intended use.

Based on the totality of the data and information described above, Oobli concludes that brazzein preparation is GRAS for its intended use.

Standards of Identity

In the notice, Oobli states its intention to use brazzein 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.

Potential Labeling Issues

Under section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act), a food is misbranded if its labeling is false or misleading in any way. Section 403(r) of the FD&C Act lays out the statutory framework for labeling claims characterizing a nutrient level in a food or the relationship of a nutrient to a disease or health-related condition (also referred to as nutrient content claims and health claims). If products containing brazzein preparation bear any nutrient content or health claims on the label or in labeling, such claims are subject to the applicable requirements and are under the purview of ONFL in the Nutrition Center of Excellence. OPMAS did not consult with ONFL on this issue or evaluate any information in terms of labeling claims. Questions related to food labeling should be directed to ONFL.

Section 301(ll) of the 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 Oobli's supplement concluding that brazzein 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 brazzein preparation. Accordingly, our response should not be construed to be a statement that foods containing brazzein preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusions

Based on the information that Oobli provided, as well as other information available to FDA, we have no questions at this time regarding Oobli's conclusion that brazzein preparation is GRAS under its intended conditions of use. This letter is not an affirmation that brazzein 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 the supplement to GRN 001142 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

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Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
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References

1. 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

2. Renwick, A.G. 2008. The use of a sweetener substitution method to predict dietary exposures for the intense sweetener rebaudioside A. Food and Chemical Toxicology 46:S61–S69.