



Erik Hedrick, Ph.D.
Burdock Group Consultants
859 Outer Road
Orlando, FL 32814

Re: GRAS Notice No. GRN 001202

Dear Dr. Hedrick:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001202. We received the notice that you submitted on behalf of Impossible Foods Inc. (IFI) on June 20, 2024, and filed it on August 15, 2024. IFI submitted amendments to the notice on November 18, 2024, February 5, 2025, and February 27, 2025, that provided additional information on the intended use, method of manufacture, specifications, dietary exposure, and safety of the notified substance.

The subject of the notice is soy leghemoglobin preparation from a strain of *Komagataella phaffii*¹ for use as an ingredient to impart flavor and provide a source of iron in plant-based meat, poultry, and fish analogue products;² nutrition bars; and protein and nutritional powders at levels that provide up to 2% soy leghemoglobin protein in the finished food³ (Table 1). The notice informs us of IFI's view that these uses of soy leghemoglobin preparation are GRAS through scientific procedures.

Table 1. Intended food categories and maximum use levels for soy leghemoglobin protein

Food Categories (all categories refer to plant-based analogue products)	Maximum intended use level (%)
Beef	1.0
Pork	0.4
Lamb, goat, game meat	2.0
Liver and organ meat	2.0
Chicken, whole pieces	0.1

¹ *Komagataella phaffii* was previously classified as *Pichia pastoris* (Kurtzman, C.P. (2009) Biotechnological strains of *Komagataella (Pichia) pastoris* are *Komagataella phaffii* as determined from multigene sequence analysis. *Journal of Industrial Microbiology and Biotechnology*. 36(11):1435-1438.)

² The intended uses of soy leghemoglobin include various mixed dishes, i.e., foods that are mixtures of component foods including meat and poultry analogues. IFI clarified that the maximum intended use levels for mixed dishes are on the basis of the meat analogue component rather than the whole mixture.

³ IFI states that soy leghemoglobin protein is not intended for use in infant formula, products under the jurisdiction of the United States Department of Agriculture, or in foods for which standards of identity preclude its use. Food categories that include terms such as meat, beef, chicken, pork, goat, turkey, duck, lamb, poultry, seafood, and fish refer to plant-based (i.e., non-meat) alternative products that IFI expects would be consumed in a manner similar to these conventional foods.

Turkey, duck, and other poultry	0.5
Fish	0.4
Eggs and omelets	0.12
Cold cuts and cured meat	0.5
Bacon	0.5
Frankfurters	0.4
Sausages	0.6
Meat mixed dishes	1.0
Poultry mixed dishes	0.1
Seafood mixed dishes	0.4
Egg rolls, dumplings, sushi	0.2
Burritos and tacos	0.2
Nachos	0.2
Other Mexican mixed dishes	0.2
Pizza	0.17
Burgers	0.6
Frankfurter sandwiches	0.24
Chicken and turkey sandwiches	0.3
Egg/breakfast sandwiches	0.0375
Seafood sandwiches	0.24
Other sandwiches	0.45
Soups	0.55
Nutrition bars	2.0
Protein and nutritional powders	2.0

Our use of the term, “soy leghemoglobin 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 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 (NCE). The Office of Pre-Market Additive Safety (OPMAS) did not consult with ONFL regarding the appropriate common or usual name for “soy leghemoglobin preparation.”

IFI describes soy leghemoglobin preparation as a mixture containing soy leghemoglobin protein, *K. phaffii* proteins, sodium chloride, and sodium ascorbate. The preparation is produced using *K. phaffii* MXYO291, which was constructed from the commercially available *K. phaffii* BG11. IFI describes *K. phaffii* as a non-pathogenic, non-toxicogenic, and well-characterized yeast with a history of safe use in the food industry. IFI states that the production organism is constructed through transformation of the recipient BG11 strain, including the insertion of a codon-optimized gene encoding the leghemoglobin LGB2 apoprotein from soybean (*Glycine max*). IFI states that the

production organism is the same as described previously in GRN 000737.⁴ The production organism expresses the soy leghemoglobin apoprotein and the yeast heme B prosthetic group, which combine to form soy leghemoglobin protein. IFI sequenced the *K. phaffii* production organism genome and states that the production organism does not contain antibiotic resistance genes.

IFI describes the method of manufacture for soy leghemoglobin. IFI states that soy leghemoglobin preparation is manufactured by submerged fed-batch fermentation of the *K. phaffii* production organism under controlled conditions. The culture is periodically tested to ensure production strain identity, purity, and protein generating ability. Following fermentation, the *K. phaffii* cells are lysed by mechanical shearing or high-pressure homogenization. Insoluble content in the lysate is removed by centrifugation and microfiltration. The resulting lysate is concentrated by ultrafiltration and subjected to a heat treatment step to inactivate any residual *K. phaffii* cells. The resulting concentrate is stabilized with sodium chloride and sodium ascorbate and stored frozen. The frozen concentrate is standardized to a final concentration of 2 to 15% soy leghemoglobin protein. IFI describes an additional optional step in which the soy leghemoglobin preparation is spray dried.⁵ IFI states that all raw materials are food-grade and approved for their respective uses via a regulation in Part 21 of the U.S. Code of Federal Regulations, are the subject of an effective food contact notification, or are GRAS for the intended use. Further, IFI notes that the manufacturing process is performed in accordance with current good manufacturing practices and that the components of the fermentation media are not derived from major food allergens.

IFI provides specifications for soy leghemoglobin preparation and states that the specifications meet those established in the Food Chemicals Codex (FCC, 14th Edition) and 21 CFR 73.520 for soy leghemoglobin. Specifications include the content of soy leghemoglobin protein (2 to 15% weight/weight (w/w))⁶ that has a purity of $\geq 65\%$ w/w, total protein ($>10\%$ w/w), fat ($\leq 2\%$ w/w), moisture ($\geq 74\%$ w/w), carbohydrates ($\leq 6\%$ w/w), ash ($\leq 4\%$ w/w), solids ($\leq 26\%$ w/w), pH (6.0 to 9.7), lead (< 0.05 mg/kg), arsenic (< 0.05 mg/kg), mercury (< 0.05 mg/kg), and cadmium (< 0.05 mg/kg)⁷, as well as limits for microorganisms. IFI provides results from the analyses of five non-consecutive batches to demonstrate soy leghemoglobin preparation can be

⁴ The subject of GRN 000737 is soy leghemoglobin preparation from a strain of *Pichia pastoris* for use at a level up to 0.8% soybean leghemoglobin protein to optimize flavor in ground beef analogue products intended to be cooked. FDA evaluated GRN 000737 and responded in a letter dated July 23, 2018, stating that the agency had no questions at that time regarding the notifier's GRAS conclusion.

⁵ IFI states that the optional spray drying step is not currently used, but IFI states that the spray-drying step would result in a soy leghemoglobin preparation with a different composition, including reduced moisture content and higher protein content. Therefore, the intended use levels for the spray-dried soy leghemoglobin preparation would be lower to achieve the same intended use levels of soy leghemoglobin protein in the specified food categories.

⁶ IFI states that the content of soy leghemoglobin protein in the preparation may exceed 15 % if additional water is removed during the manufacturing process, but IFI notes that the removal of additional water would not change the composition of the dry solids fraction of the soy leghemoglobin preparation.

⁷ In an amendment dated February 27, 2025, IFI discusses their intention to revise the specifications for lead from < 0.4 mg/kg to < 0.05 mg/kg and for cadmium from < 0.2 mg/kg to < 0.05 mg/kg by the end of 2025. The results from the batch analyses provided in the notice demonstrate that soy leghemoglobin preparation already meets the revised specifications.

manufactured to meet these specifications.

IFI provides estimates of dietary exposure to soy leghemoglobin protein and soy leghemoglobin preparation based on the intended uses and food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES). IFI estimates the mean and 90th percentile eaters-only dietary exposure based on a conservative assumption that consumers will substitute meat analogue products containing soy leghemoglobin preparation for traditional meat products on a 1:1 basis. IFI estimates the mean and 90th percentile eaters-only dietary exposure to soy leghemoglobin protein for the U.S. population aged 1 year and older to be approximately 0.8 g/person (p)/d (12 mg/kg body weight (bw)/d) and 1.8 g/p/d (28 mg/kg bw/d), respectively. IFI provides estimates of dietary exposure to soy leghemoglobin preparation on the hydrated and dry solids basis using the estimates of dietary exposure to soy hemoglobin protein and the average protein content of the preparation. IFI states that the hydrated soy hemoglobin preparation contains on average 11.7% of soy leghemoglobin protein; therefore, IFI estimates the mean and the 90th percentile dietary exposure to the hydrated preparation for the U.S. population aged 1 year and older to be 102.7 and 237.8 mg/kg bw/d, respectively. Similarly, IFI states that the preparation contains on average 17% of soy leghemoglobin protein on a dry solids basis; therefore, IFI estimates the mean and the 90th percentile dietary exposure to the dry solids portion of the preparation for the U.S. population aged 1 year and older to be 17.5 and 40.4 mg/kg bw/d, respectively.

IFI provides an estimate of dietary exposure to iron from the intended uses of soy leghemoglobin protein based on the 90th percentile dietary exposure to soy leghemoglobin protein described above and that each molecule of soy leghemoglobin protein contains one molecule of heme and one molecule of iron. IFI estimates the 90th percentile dietary exposure to iron for the U.S. population aged 1 year and older to be 6.3 mg/p/d.

IFI discusses the safety of the intended use of soy leghemoglobin preparation by discussing the history of safe use of the host organism *K. phaffii*, the safety of oral exposure to *K. phaffii* proteins, and the safety of consumption of soy leghemoglobin protein. IFI also notes that the safety of *K. phaffii*, that of *K. phaffii* proteins that co-purify with soy leghemoglobin, and the safety of soy leghemoglobin protein were previously discussed in GRN 000737.⁴ For the current GRAS notice, IFI performed additional studies, such as published 14-day and 90-day feeding studies, genotoxicity studies, and pepsin digestibility studies. In the published 90-day dietary study, no adverse effects were reported at up to 4,798.3 mg/kg bw/d and 5,761.5 mg/kg bw/d soy leghemoglobin preparation for male and female rats respectively. IFI states that soy leghemoglobin preparation is non-mutagenic and non-clastogenic. Potential allergenicity of proteins in the soy leghemoglobin preparation were also evaluated using literature review, bioinformatics sequence comparisons to known allergens or toxins, and a published *in vitro* pepsin digestion study. Analysis found no evidence that soy leghemoglobin protein or residual *K. phaffii* proteins in the soy leghemoglobin preparation could pose any significant risk of allergenicity or toxicity to consumers. IFI states that the level of iron consumption from the intended uses of soy leghemoglobin

preparation is well under the tolerable upper intake level for iron that is established by the Institute of Medicine and within the recommended daily allowance for all non-infant age groups and sexes. IFI conducted a literature search through November 2023 and did not identify any information that would contradict its GRAS conclusion.

Based on the information presented in the notice, IFI concludes that soy leghemoglobin preparation is GRAS under the conditions of its intended use.

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 soy leghemoglobin 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 NCE. The 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.

Allergen Labeling

The FD&C Act requires that the label of a food that is or contains an ingredient that contains a “major food allergen” declare the allergen’s presence (section 403(w)). The FD&C Act defines a “major food allergen” as one of nine foods or food groups (i.e., milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame) or a food ingredient that contains protein derived from one of those foods. Soy leghemoglobin preparation requires labeling under the FD&C Act because it contains protein derived from soybean.

Potential Requirement for a Color Additive Petition

There is no GRAS provision for color additives. Soy leghemoglobin preparation is reddish brown in color. As such, the intended use of soy leghemoglobin preparation as described in GRN 001202 may constitute a color additive use under section 201(t)(1) of the FD&C Act and FDA’s implementing regulations in 21 CFR Part 70. Under section 201(t)(1) and 21 CFR 70.3(f), a color additive is a material that is a dye, pigment, or other substance made by a synthetic process or similar artifice, or is extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source. Under 21 CFR 70.3(g), a material that otherwise meets the definition of a color additive can be exempt from that definition if it is used (or is intended to be used) solely for a purpose or purposes other than coloring. Our response to GRN 001202 is not an approval for use as a color additive nor is it a finding of the Secretary of the Department of Health and Human Services within the meaning of section 721(b)(4) of the FD&C Act. Questions about color additives should be directed to the Division of Food Ingredients in OPMAS.

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

Conclusions

Based on the information that IFI provided, as well as other information available to FDA, we have no questions at this time regarding IFI's conclusion that soy leghemoglobin preparation is GRAS under its intended conditions of use. This letter is not an affirmation that soy leghemoglobin 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 GRN 001202 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

**Susan J.
Carlson -S**

Digitally signed by Susan
JCarlson -S

Date: 2025.03.07 17:47:38
-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