



Tomer Gold  
Imagindairy Ltd.  
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3508504, ISRAEL

Re: GRAS Notice No. GRN 001145

Dear Mr. Gold:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001145. We received Imagindairy Ltd.'s (Imagindairy) notice on May 15, 2023 and filed it on August 21, 2023. Imagindairy submitted an amendment to the notice on October 26, 2023, that clarified the manufacturing process, specifications, intended uses, dietary exposure, and provided additional information on the production strain and safety.

The subject of the notice is  $\beta$ -lactoglobulin produced by *Aspergillus oryzae* "Ao\_st0002" for use as a source of protein at the maximum levels shown in Table 1.<sup>1</sup> The notice informs us of Imagindairy's view that these uses of the  $\beta$ -lactoglobulin are GRAS through scientific procedures.

Table 1. Proposed uses and use levels for  $\beta$ -lactoglobulin

Food Category	Food Use	Maximum $\beta$ -Lactoglobulin Use Levels
Nutritional Products	Meal replacements and supplements	15%
	Powdered nutritional beverages	25%
	Nutritional bars	35%
	Electrolyte-type sports drinks	6%
	Performance nutritional beverages, high protein	25%
Dairy and Dairy-based Products	Fluid milk, powdered milk, flavored milk, milk-based drinks and drink mixes (e.g., dairy smoothies, hot chocolate from mix), milk alternatives	6%
	Cream, half and half, cream cheese, cheese spread, whipped cream	15%
	Yogurt and fermented milk products	8%

<sup>1</sup> Imagindairy states that  $\beta$ -lactoglobulin produced by *A. oryzae* "Ao\_st0002" is not intended for use in infant formula or in any products under the jurisdiction of the United States Department of Agriculture.

Food Category	Food Use	Maximum $\beta$ -Lactoglobulin Use Levels
	Spreads, dips	10%
	Cream substitutes	15%
	Frozen dairy desserts and mixes	10%
	Cheese used primarily as ingredients (e.g., ricotta cheese)	15%
	Semi-hard cheese (e.g., feta, Camembert, brie)	25%
	Desserts and mousses	5%
Sugar-based Products	Confections (including chocolate confections)	10%
	Coatings and fillings	10%
Dressings	Salad dressings	5%
	Minor main entrée sauces (e.g., Alfredo sauce, white sauce, cheese sauce)	6%
Baked Goods	French toast, crepes, pancakes, bagels, scones, biscuits, croissants	10%
	Breads and rolls, English muffins, pizza crust	10%
	Doughnuts, toaster pastries, muffins	10%
	Cookies and brownies, crackers, popcorn, potato chips, tortilla chips, hard pretzels/snack mix	5%
Egg Products	Egg substitutes	10%

Imagindairy describes  $\beta$ -lactoglobulin produced by fermentation from *A. oryzae* "Ao\_st0002" as a white to off-white to yellowish powder containing  $\geq 60\%$  protein, with the  $\beta$ -lactoglobulin protein comprising  $\geq 85\%$  of the total protein content.  $\beta$ -lactoglobulin is a major whey protein component of bovine milk and has a concentration in bovine milk ranging from 2–3 g/L, which represents approximately 9% of the total milk protein content. Imagindairy states that  $\beta$ -lactoglobulin produced by fermentation from *A. oryzae* "Ao\_st0002" has a molecular weight of approximately 18 kDa and a CAS registry number of 9066-45-9.

Imagindairy describes the production organism used in the manufacture of  $\beta$ -lactoglobulin. Imagindairy states that *A. oryzae* "Ao\_st0002" is nonpathogenic and nontoxigenic. *A. oryzae* "Ao\_st0002" is genetically engineered to produce  $\beta$ -lactoglobulin from the host strain, *A. oryzae* "Ao\_st0044," which was constructed from the base strain *A. oryzae* RIB40 that has been deposited in an international culture collection, the World Data Centre for Microorganisms (WDCM) with an accession number WDCM 139. Imagindairy states that the production organism was constructed through the chromosomal-integration of an expression cassette carrying a *de novo* synthesized, codon-optimized gene sequence that encodes  $\beta$ -lactoglobulin protein with

an amino acid sequence that is identical to the amino acid sequence of a mature  $\beta$ -lactoglobulin isoform B protein from domestic cow (UniProt accession number: P02754). Imagindairy states that the inserted DNA is stably integrated into the genome of the production organism and is confirmed by sequencing the expression cassette and determining the copy number after 38 generations of growth on solid media. Imagindairy states that the production organism, *A. oryzae* “Ao\_st0002,” does not contain any antibiotic resistance genes and does not produce any undesirable secondary metabolites. Imagindairy states that *A. oryzae* “Ao\_st0002” is not detected in the final product.

Imagindairy describes the manufacture of  $\beta$ -lactoglobulin by precision fermentation of a genetically engineered *A. oryzae* “Ao\_st0002.” The fermentation process begins with inoculating a spore suspension stock into the seed fermentation to increase the biomass amount. After the seed fermentation, the culture is transferred to the main fermentation to produce  $\beta$ -lactoglobulin. At the end of the fermentation, the biomass is separated from the broth containing  $\beta$ -lactoglobulin and the resulting crude  $\beta$ -lactoglobulin then undergoes a series of purification steps including optional pH, conductivity, and temperature adjustment; centrifugation and/or filtration to remove impurities; concentration and dialysis using ultrafiltration/diafiltration; sterile filtration; and spray drying to obtain the final  $\beta$ -lactoglobulin. Imagindairy states that  $\beta$ -lactoglobulin is manufactured according to current good manufacturing practices and that all raw materials, processing aids, filtration aids, and pH adjusters are food grade, high-quality chemical or pharmaceutical grades. Imagindairy states that all raw materials and processing aids used in the manufacturing process are used in accordance with appropriate U.S. regulations, are GRAS for their intended use, or are the subject of an effective food contact notification.

Imagindairy provides specifications for  $\beta$ -lactoglobulin that include total protein ( $\geq$  60%),  $\beta$ -lactoglobulin ( $\geq$  85% total protein), and limits for moisture ( $\leq$  10%), ash ( $\leq$  10%), fat ( $\leq$  5%), total carbohydrates ( $\leq$  30%), pH (6-8), heavy metals, including lead ( $\leq$  0.1 mg/kg), and microorganisms, including *Salmonella* serovars (absent in 25 g). Imagindairy provides the results from the analyses of three non-consecutive batches to demonstrate that  $\beta$ -lactoglobulin can be manufactured to meet these specifications.

Imagindairy states that the intended uses of  $\beta$ -lactoglobulin are substitutional for the uses described in GRNs 000863 and 001056<sup>2</sup> and cites the estimates of the cumulative dietary exposure to  $\beta$ -lactoglobulin from GRN 001056. Imagindairy states that the eaters-only cumulative dietary exposure to  $\beta$ -lactoglobulin is estimated to be 31.5 g/person (p)/d at the mean and 57.6 g/p/d at the 90<sup>th</sup> percentile for the U.S. population aged 2 years and older based on food consumption data from the 2015-2018 National Health and Nutrition Examination Survey (NHANES).

Imagindairy states that milk and milk protein have a long safe history of use as foods.

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<sup>2</sup> The subjects of GRNs 000863 and 001056 are  $\beta$ -lactoglobulin produced by *Trichoderma reesei* “QM6a-PD1” and  $\beta$ -lactoglobulin produced by *Komagataella phaffii* “yRMK-66.” We evaluated these notices and responded in letters dated March 25, 2020 and February 15, 2023, respectively, stating that we had no questions at that time regarding the notifiers’ GRAS conclusions.

Imagindairy notes that  $\beta$ -lactoglobulin from bovine milk is a small protein that is relatively pepsin-resistant in the stomach but is rapidly digested in the intestine. A published study shows that  $\beta$ -lactoglobulin is not genotoxic or mutagenic and shows no toxicity at doses up to 1,000 mg/kg body weight/d, the highest dose tested in a subchronic oral toxicity study. Except for certain sensitive populations (e.g., milk-allergic and lactose-intolerant individuals),  $\beta$ -lactoglobulin, as a milk protein, is not known to have adverse effects in consumers. Imagindairy discusses the safety of *A. oryzae*-expressed  $\beta$ -lactoglobulin by discussing the safety of *A. oryzae* as an organism that has been long consumed as part of fermented foods such as miso and sake, safety of the host strain of *A. oryzae* “Ao\_st0002” expressing  $\beta$ -lactoglobulin, and safety of the  $\beta$ -lactoglobulin protein. Imagindairy states that *A. oryzae* is not considered to be pathogenic to humans, and the safety and low risk associated with the commercial use of *A. oryzae* has long been well established. Imagindairy also states that based on the use of widely practiced genetic modification techniques to produce the host strain, stability of the genetically integrated expression cassette, and the absence of selection markers and origin of replication sequences, the strain *A. oryzae* “Ao\_st0002” is also safe for use in the production of  $\beta$ -lactoglobulin. Imagindairy did a literature search through April 2023 and did not identify any new information that could contradict its conclusion that the intended use of  $\beta$ -lactoglobulin is safe and GRAS.

Based on the totality of information, Imagindairy concludes that  $\beta$ -lactoglobulin produced by *A. oryzae* “Ao\_st0002” is GRAS for its intended use.

## **Standards of Identity**

In the notice, Imagindairy states its intention to use  $\beta$ -lactoglobulin in several food categories, including foods for which standards of identity exist, located in Title 21 of the Code of Federal Regulations. 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, & Cosmetic (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  $\beta$ -lactoglobulin 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 Office of Nutrition and Food Labeling (ONFL) in CFSAN. Office of Food Additive Safety (OFAS) 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.  $\beta$ -lactoglobulin produced by *A. oryzae* “Ao\_stooo2” requires labeling under the FD&C Act because it contains protein derived from milk.

### **Section 301(l) of the FD&C Act**

Section 301(l) 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(l)(1)-(4) applies. In our evaluation of Imagindairy’s notice concluding that  $\beta$ -lactoglobulin is GRAS under its intended conditions of use, we did not consider whether section 301(l) or any of its exemptions apply to foods containing  $\beta$ -lactoglobulin. Accordingly, our response should not be construed to be a statement that foods containing  $\beta$ -lactoglobulin, if introduced or delivered for introduction into interstate commerce, would not violate section 301(l).

### **Conclusions**

Based on the information that Imagindairy provided, as well as other information available to FDA, we have no questions at this time regarding Imagindairy’s conclusion that  $\beta$ -lactoglobulin is GRAS under its intended conditions of use. This letter is not an affirmation that  $\beta$ -lactoglobulin 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 001145 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,

Susan J.  
Carlson -S

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