Brian P. Sylvester
Covington & Burling LLP
One CityCenter
850 Tenth Street, NW
Washington, DC 20001

Re: GRAS Notice No. GRN 001056

Dear Mr. Sylvester:

The Food and Drug Administration (FDA, we) completed our evaluation of GRN 001056. We received the notice that you submitted on behalf of Remilk Ltd. (Remilk) on March 8, 2022, and filed it on June 30, 2022. Remilk submitted amendments to the notice on October 12, 2022, and November 30, 2022, providing information about the production organism, clarifying information regarding the specifications, and an updated literature search discussing the safety of the production organism.

The subject of the notice is β-lactoglobulin produced by *Komagataella phaffii* strain "yRMK-66"[1] (*K. phaffii* "yRMK-66") for use as a source of protein at levels ranging from 5 to 35% in food. [2] The intended uses and use levels are shown in Table 1. The notice informs us of Remilk's view that these uses of β-lactoglobulin are GRAS through scientific procedures.

Table 1. Proposed uses and use levels for β-lactoglobulin

Food Category	Food Uses	Maximum Use Level (%)
Nutritional products	Meal replacements and supplements	15
	Powdered nutritional beverages	25
	Electrolyte-sports drinks	6
	Performance nutritional beverages, high protein	25
	Nutritional bars	35
Dairy and dairy-based products	Milk, powdered milk, flavored milk, milk substitutes	6
	Cream, half and half, cream cheese, whipped cream	15
	Spreads, dips	10

[1] Remilk states that *Pichia pastoris* was reclassified as *K. phaffii* as reported in Kurtzman (Ref. 1).

[2] Remilk states that β-lactoglobulin produced by *K. phaffii* "yRMK-66" is not intended for use in infant formula or in any products under the jurisdiction of the U.S. Department of Agriculture.

Food Category	Food Uses	Maximum Use Level (%)
	Cream substitutes	15
	Yogurt and fermented milk products	8
	Ice cream, frozen yogurt	8
	Cheese used as ingredient	15
	Semi-hard cheese	25
	Mousses and desserts	5
Sugar-based products	Confections (including chocolate)	10
	Coatings and fillings	10
Baked goods	Cookies, brownies	5
	Doughnut, toaster pastries, muffins	10
	French toast, crepes, pancakes, bagels, scones, biscuits, croissants	10
	Bread, rolls, English muffins, pizza crust	10
	Crackers, popcorn, tortilla chips, hard pretzels, snack mix	5
Dressings	Creamy salad dressings	5
	Minor entrée sauces (e.g., Alfredo sauce)	6
Egg products	Egg substitutes	10

Our use of the term, "β-lactoglobulin," 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 Center for Food Safety and Applied Nutrition (CFSAN). The Office of Food Additive Safety (OFAS) did not consult with ONFL regarding the appropriate common or usual name for "β-lactoglobulin."

Remilk describes β-lactoglobulin as a white to off-white powder containing at least 70% protein, with β-lactoglobulin comprising ≥80% of the protein content. β-lactoglobulin is a major whey protein of ruminant species and has a concentration in bovine milk ranging from 2–3 g/L, which represents 7–9% of the total milk protein content. Remilk states that β-lactoglobulin produced by fermentation from *K. phaffii* "yRMK-66" has a molecular weight of approximately 18 kDa and a CAS registry number of 9066-45-9.

Remilk describes the production organism used in the manufacture of β-lactoglobulin. Remilk states that *K. phaffii* "yRMK-66" is non-pathogenic and non-toxigenic, and that the strain's identity was confirmed using whole genome sequencing. *K. phaffii* "yRMK-66" is genetically engineered to produce β-lactoglobulin from the host strain, *K. phaffii* strain NRRL Y-11430. Remilk 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 β-lactoglobulin identical to bovine β-lactoglobulin from domestic cow (*Bos taurus*). Remilk states that the integration stability of the β-lactoglobulin gene sequence was confirmed by reverse transcription-quantitative polymerase chain reaction over the course of three independent fermentation processes, while the stability of β-lactoglobulin production was assessed through a colorimetric quantification assay. Remilk states that the production organism does not contain any antibiotic resistance genes or mobile genetic elements and is not capable of DNA transfer to other organisms.

Remilk states that β-lactoglobulin is manufactured through fermentation of the production organism, *K. phaffii* "yRMK-66." The β-lactoglobulin is secreted into the fermentation medium and then separated from the biomass by series of filtration steps, concentrated via ultrafiltration and diafiltration, followed by drying and packaging. Remilk states that the β-lactoglobulin is manufactured in accordance with current good manufacturing practices and that all raw materials, processing aids, filtration aids and pH adjusters are food grade and approved for their respective uses in accordance with an appropriate US regulation, are the subject of an effective food contact notification, or are GRAS for their intended use. Remilk states that none of the raw materials used during manufacturing are derived from major allergens.

Remilk provides specifications for β-lactoglobulin that include protein content (≥70% w/w), β-lactoglobulin as percent of the total protein content (≥80% w/w), moisture (≤7% w/w), ash (≤4% w/w), fat (≤4% w/w), total carbohydrates (≤20% w/w), pH (5.0–7.5), limits for heavy metals, including lead (≤0.1 mg/kg), and limits for microorganisms, including *Salmonella* serovars (absent in 25 g). Remilk provides the results from the analyses of three non-consecutive batches to demonstrate that β-lactoglobulin can be manufactured to meet the stated specifications.

Using food consumption data from the 2015-2018 National Health and Examination Survey (NHANES), Remilk estimates the eaters-only dietary exposure to β-lactoglobulin from the proposed uses to be 31 g/person (p)/d at the mean and 56.4 g/p/d at the 90th percentile for the U.S. population aged 2 years and older. In addition, Remilk estimates an eaters-only cumulative dietary exposure to β-lactoglobulin from all current and proposed uses of β-lactoglobulin to be 31.5 g/p/d at the mean and 57.6 g/p/d at the 90th percentile for the U.S. population aged 2 years and older. Remilk states that the proposed uses of β-lactoglobulin are substitutional for other protein sources and therefore, there will not be an increase in the overall dietary exposure to protein.

Remilk states that the safety of β-lactoglobulin produced by *K. phaffii* "yRMK-66" is supported by the long history of safe consumption of bovine milk and milk-derived proteins in human foods, as β-lactoglobulin is a component of milk.

Remilk corroborates the safety of its β-lactoglobulin by incorporating the safety data

from GRN 000863[3]. Remilk states that β-lactoglobulin produced by *K. phaffii* "yRMK-66" is identical to the commercially available bovine-produced β-lactoglobulin present in cow milk, which has a long history of safe consumption from dairy sources. Remilk further states that, in a survey of the literature, there were no reported adverse effects associated with the consumption of concentrated milk proteins, except for certain sensitive populations such as milk-allergic individuals.

Remilk discusses the allergenic potential of residual *K. phaffii* proteins that remain in the final product following manufacturing. Remilk states that all of the yeast proteins are present in low amounts (<0.15%) and are unlikely to pose a risk to consumers. Remilk incorporates by reference relevant safety data on the production organism, *K. phaffii,* from GRNs 000204, 000737, 000967, and 001001,[4] to corroborate the safety of *K. phaffii* "yRMK-66." Remilk states that *K. phaffii* has a long history of safe use in food production. Remilk discusses the allergenic potential posed by β-lactoglobulin produced by *K. phaffii* "yRMK-66." Remilk states that β-lactoglobulin produced by *K. phaffii* "yRMK-66" is identical to β-lactoglobulin found in bovine milk and isolated bovine milk proteins, and will produce a milk protein allergy when consumed by milk-allergic individuals.

Based on the available data and information, Remilk concludes that β-lactoglobulin produced by *K. phaffii* "yRMK-66" is GRAS for its intended use.

Standards of Identity

In the notice, Remilk states its intention to use β-lactoglobulin 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 β-lactoglobulin bear any nutrient content or health claims on the label or in labeling, such

[3] The subject of GRN 000863 is β-lactoglobulin produced by *Trichoderma reesei* strain "QM6a-PD1." We evaluated this notice and responded in a letter dated March 25, 2020, stating that we had no questions at that time regarding the notifier's GRAS conclusion.

[4] The subjects of GRNs 000204, 000737, 000967, and 001001 are phospholipase C enzyme preparation from *K. phaffii* expressing a heterologous phospholipase C gene, soy leghemoglobin preparation produced by *K. phaffii* strain "MXY0291", soluble egg-white protein produced by *K. phaffii* strain GSD-1209, and myoglobin preparation produced by *K. phaffii* strain "t838417", respectively. We evaluated these notices and responded in letters respectively dated December 5, 2006, July 23, 2018, September 9, 2021, and December 3, 2021, stating that we had no questions at that time regarding the notifiers' GRAS conclusions.

claims are subject to the applicable requirements and are under the purview of ONFL in CFSAN. 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. β-lactoglobulin produced by *K. phaffii* "yRMK-66" requires labeling under the FD&C Act because it contains protein derived from milk.

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

Conclusions

Based on the information that Remilk provided, as well as other information available to FDA, we have no questions at this time regarding Remilk's conclusion that β-lactoglobulin is GRAS under its intended conditions of use. This letter is not an affirmation that β-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 001056 is accessible to the public at www.fda.gov/grasnoticeinventory.

Sincerely,

Susan J. Carlson -S

Digitally signed by Susan J. Carlson -S
Date: 2023.02.15 11:14:37 -05'00'

Susan J. Carlson, Ph.D.
Director
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition

Reference

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