

Executive summary: APPLICATION FOR THE AUTHORISATION OF *Rhizomucor pusillus* mycelium AS A NOVEL FOOD INGREDIENT

Food Standards Australia New Zealand

Applicant: The Protein Brewery B.V., Goeseelsstraat 10, 4817 MV Breda, The Netherlands.

This novel food dossier contains the application for *Rhizomucor pusillus* mycelium to gain pre-market authorization in the Australian New Zealand market in accordance with Standard 1.5.1 (Novel Foods) of the Australia New Zealand Food Standards Code and the requirements of Food Standards Australia New Zealand (FSANZ). Based on the FSANZ novel food classification framework, *Rhizomucor pusillus* mycelium is considered a Category C.6 novel food, namely food ingredients derived from a new source.

Rhizomucor pusillus mycelium is a dried fungal mycelium product obtained via fermentation led by a filamentous fungus verified according to The Index Fungorum. Its main components include proteins and dietary fibers. Furthermore, nutritionally relevant inherent constituents of *Rhizomucor pusillus* mycelium include amino acids, minerals, vitamins and fatty acids.

Rhizomucor spp. are widely used for the production of microbial rennin-like milk clotting enzymes and are commonly found in fermented foods outside Australia and New Zealand. In the EU, food enzymes produced from the same fungal species are considered safe for food production. *Rhizomucor pusillus* mycelium has a self-affirmed GRAS status in the USA and is authorized as a novel food in the European Union and Singapore.

The production process complies with Hazard Analysis and Critical Control Points (HACCP) principles and food safety requirements in accordance with the Australia New Zealand Food Standards Code, including the Food Safety Standards (Chapter 3) and contaminant limits established under Standard 1.4.1 and Schedule 19 (Maximum Levels of Contaminants and Natural Toxicants).

Rhizomucor pusillus mycelium is targeted at the general population and is intended for use as a protein- and fibre-rich ingredient in a range of conventional food categories at varying use levels. In addition, *Rhizomucor pusillus* mycelium may be used in special purpose foods, including meal replacement products, sports nutrition products, supplements and other foods formulated to meet specific nutritional requirements, at specific use levels.

The safety of the *Rhizomucor pusillus* mycelium fungus is assessed based on taxonomic classification and complete strain characterization through whole-genome sequence analysis. *In silico* studies revealed that the fungus is not capable to produce mycotoxins, antibiotics, or virulence factors. This was confirmed by demonstrating that the *Rhizomucor pusillus* mycelium fungus does not produce antibiotic substances against selected bacterial strains and does not produce mycotoxins. There is no reason to believe that *Rhizomucor pusillus* mycelium ingestion may be a risk for invasive infection as the fungus is not viable in *Rhizomucor pusillus* mycelium and there are no spores in the final product. Analyses and scientific studies show no safety

concerns regarding heavy metals, mycotoxins, or microorganisms in *Rhizomucor pusillus* mycelium. No antinutritional factors or undesired compounds were identified.

Toxicology studies (including *in-vitro* genotoxicity studies and a sub-chronic 90-day rat study) have been conducted to support the safety of *Rhizomucor pusillus* mycelium. There was no evidence of genotoxicity in the *in vitro* tests, and the highest dose tested in the 90-day study was established as the no-observed-adverse-effect-level (NOAEL).

In silico allergenicity studies revealed homology between *Rhizomucor pusillus* mycelium fungal proteins and minor allergenic proteins with low clinically relevance from food sources requiring allergen labeling. These minor allergenic proteins, however, shared a greater degree of sequence homology with common foods and human proteins, indicating evolutionary conservation. *In vitro* IgE studies confirm a low risk of cross-reactive allergies. Although it cannot be excluded that on a rare occasion (immunocompromised individuals) people may show an allergenic reaction to *Rhizomucor pusillus* mycelium, the overall allergenic potential of *Rhizomucor pusillus* mycelium is considered to be low.

When maximum use levels are observed, no adverse nutritional effects are expected from the habitual use of *Rhizomucor pusillus* mycelium in the Australian New Zealand dietary context. We conclude that *Rhizomucor pusillus* mycelium is safe as a food ingredient under the proposed conditions of use and intake levels.