



Joanne Donoghue  
Director, EHS & Operations  
Mascoma LLC  
67 Etna Road, Suite 200  
Lebanon, New Hampshire, 03766

Re: GRAS Notice No. GRN 000842

Dear Ms. Donoghue:

The Food and Drug Administration (FDA, we) has completed its evaluation of GRN 000842. We received Mascoma LLC's (Mascoma)'s GRAS notice, dated January 15, 2019, on February 6, 2019, and filed it on March 29, 2019.

The subject of the notice is “genetically engineered maltogenic alpha-amylase enzyme produced by *Saccharomyces cerevisiae* carrying a maltogenic alpha-amylase gene from *Geobacillus stearothermophilus*” (maltogenic alpha-amylase enzyme preparation) as an enzyme used at a maximum level of 164 mg Total Organic Solids (TOS)/kg in flour for baked goods. The notice informs us of Mascoma's view that this use of maltogenic alpha-amylase enzyme preparation is GRAS through scientific procedures.

Commercial enzyme preparations that are used in food processing contain the enzyme component that catalyzes the chemical reaction as well as substances used as stabilizers, preservatives, or diluents. Enzyme preparations may also contain components derived from the production organism and from the manufacturing process, e.g., constituents of the fermentation media or the residues of processing aids. Mascoma's notice provides information about the components in the maltogenic alpha-amylase enzyme preparation.

According to the classification system of enzymes established by the International Union of Biochemistry and Molecular Biology, maltogenic alpha-amylase is identified by the Chemical Abstracts Service number 160611-47-2 and Enzyme Commission Number 3.2.1.133.<sup>1</sup> Mascoma provides the amino acid sequence of its alpha-amylase in its notice.

Mascoma states that the *S. cerevisiae* production organism is non-pathogenic and non-toxicogenic. Mascoma describes the construction of the production strain, which includes the insertion of an expression cassette containing a genetically engineered maltogenic

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<sup>1</sup> <https://www.qmul.ac.uk/sbcs/iubmb/enzyme/EC3/2/1/133.html>

alpha-amylase gene<sup>2</sup> and the native *S. cerevisiae* promoters and terminators into the genome using targeted homologous recombination. Mascoma confirmed the integration of the gene using PCR analyses and phenotypic characterizations.<sup>3</sup> Mascoma used quantitative PCR measurement of gene copy number, qualitative PCR genotyping, and measurement of enzyme activity to confirm the stability of the integrated DNA. Mascoma states that the final production strain does not contain any antibiotic resistance genes, virulence factors, protein toxins, or enzymes involved in the synthesis of mycotoxins.

Mascoma states that maltogenic alpha-amylase enzyme preparation is manufactured starting with the fermentation of a pure culture of the *S. cerevisiae* production strain. Mascoma states that fermentation is carried out under controlled conditions and that the enzyme is produced intracellularly. After fermentation, the yeast cells are concentrated and washed *via* centrifugation. Mascoma states that the yeast cells are disrupted either by mechanical (bead mill, high-pressure homogenization, ultrasonication) or non-mechanical (physical, chemical and/or enzymatic) techniques. The broken cell suspension is either centrifuged or filtered to remove debris from the supernatant. The enzyme-containing supernatant is then concentrated and/or diafiltered to reach the desired enzyme activity. The final enzyme solution is polished, and germ filtered to remove any residual cell material and sterilize the final preparation. Finally, the enzyme is formulated with carriers such as salt, starch, or dextrin, and dried. Mascoma states that the entire process is performed using food-grade raw materials and in accordance with current good manufacturing practices. Mascoma also states that the final maltogenic alpha-amylase enzyme preparation does not contain any major food allergens from the fermentation media.

Mascoma has established food grade specifications and states that the maltogenic alpha-amylase enzyme preparation conforms to specifications established for enzyme preparations in the Food Chemicals Codex (FCC, 10<sup>th</sup> edition, 2016), and to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA, 2006). Mascoma provides analytical data from three batches of maltogenic alpha-amylase enzyme preparation to demonstrate that the manufacturing acceptance criteria can be met, including the absence of the production strain.

Mascoma intends to use maltogenic alpha-amylase enzyme preparation at a maximum level of 164 mg TOS/kg in flour for baked goods. Mascoma notes that the maltogenic alpha-amylase enzyme preparation will be deactivated during the process of baking. In estimating dietary exposure, Mascoma assumes that all the maltogenic alpha-amylase enzyme preparation will remain in the final food. Mascoma estimated dietary exposure

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<sup>2</sup> The synthetic maltogenic alpha-amylase gene is based on the publicly available Genbank sequence of the *Geobacillus stearothermophilus* and codon-optimized for *S. cerevisiae*, PCR amplified, and used in the construction of the production organism.

<sup>3</sup> Mascoma states that multiple copies for the maltogenic amylase gene are present in the production strain; the inserted DNA contains two copies of the maltogenic amylase gene and that the host strain has three copies of the chromosome into which the insert was integrated.

to maltogenic alpha-amylase enzyme preparation to be 0.614 mg TOS/kg body weight per day (kg bw/d) from the intended use.<sup>4,5</sup>

Mascoma discusses published information on the safety of the *S. cerevisiae* production organism, the safety of the donor organism *G. stearothermophilus*, the safety of microbial enzyme preparations used in food processing in general, and the safety of similar alpha-amylase enzyme preparations from other donor/production organisms.

Mascoma addresses the potential for oral allergenicity to maltogenic alpha-amylase enzyme using publicly available literature and the conclusions of several organizations and expert working groups. Further, based on bioinformatic analyses, Mascoma reports that the maltogenic alpha-amylase enzyme does not share any biologically meaningful sequence homology or sequence identity to potential oral allergens. Mascoma also states that bioinformatics searches to assess potential toxicity of maltogenic alpha-amylase enzyme did not produce any information that would indicate safety concerns. Based on the totality of the information available, Mascoma concludes that it is unlikely that oral consumption of maltogenic alpha-amylase enzyme preparations from the intended use will result in allergenic or toxic responses.

Based on the data and information summarized above, Mascoma concludes that maltogenic alpha-amylase enzyme preparation is GRAS for its intended use.

### **Standards of Identity**

In the notice, Mascoma states its intention to use maltogenic alpha-amylase enzyme preparation 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.

### **Section 301(ll) of the Federal Food, Drug, and Cosmetic Act (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 Mascoma's notice concluding that maltogenic alpha-amylase enzyme 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

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<sup>4</sup> Mascoma uses a budget method to calculate estimated dietary exposure to maltogenic alpha-amylase preparation based on an average consumption of 25 g of solid foods/kg bw/day, over a lifetime. Mascoma assumes that 50% of all solid foods (i.e., 12.5 g/kg bw/d) will be baked goods and will contain maltogenic alpha-amylase at the maximum intended use level.

<sup>5</sup> Mascoma estimates the dietary exposure to maltogenic amylase enzyme preparation to be 0.61 mg TOS/kg bw/d based on the maximum use level. FDA estimates the dietary exposure to maltogenic amylase enzyme preparation to be 0.69 mg TOS/kg bw/d based on its consumption level in the final food.

containing maltogenic alpha-amylase enzyme preparation. Accordingly, our response should not be construed to be a statement that foods containing maltogenic alpha-amylase enzyme preparation, if introduced or delivered for introduction into interstate commerce, would not violate section 301(l).

## Conclusions

Based on the information that Mascoma provided, as well as other information available to FDA, we have no questions at this time regarding Mascoma's conclusion that maltogenic alpha-amylase enzyme preparation produced by maltogenic alpha-amylase enzyme preparation produced by *Saccharomyces cerevisiae* carrying a maltogenic alpha-amylase gene from *Geobacillus stearothermophilus* is GRAS under its intended conditions of use. This letter is not an affirmation that maltogenic alpha-amylase enzyme preparation produced by maltogenic alpha-amylase enzyme preparation produced by *Saccharomyces cerevisiae* carrying a maltogenic alpha-amylase gene from *Geobacillus stearothermophilus* 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 000842 is accessible to the public at [www.fda.gov/grasnoticeinventory](http://www.fda.gov/grasnoticeinventory).

Sincerely,

Susan J. Carlso

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Susan Carlson, Ph.D.

Director

Division of Food Ingredients

Office of Food Additive Safety

Center for Food Safety

and Applied Nutrition

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