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GRAS Notice Review

May 12, 2011

Paulette M. Gaynor, Ph.D.
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Subject: GRAS Notification for Erythritol from *Yarrowia lipolytica*

Dear Dr. Gaynor:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), Baolingbao Biology Co., Ltd. hereby provides notice of a claim that the food ingredient described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to foods as a flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant, and texturizer.

Three copies of the notification are provided as required. If you have any questions or require additional information, please do not hesitate to contact me at 202-772-4953, or mmurphy@exponent.com.

Sincerely,

(b) (6)

Mary M. Murphy, MS, RD
Managing Scientist

Exponent®

Center for Chemical Regulation and Food Safety

GRAS Determination for the Use of Erythritol from *Yarrowia lipolytica* in Select Foods

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GRAS Determination for the Use of Erythritol from *Yarrowia lipolytica* in Select Foods

SUBMITTED BY:

Baolingbao Biology Co., Ltd.
NO. 1, Dongwai Huan Road
Hi-Tech Development Zone Yucheng
Shandong, China

SUBMITTED TO:

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Paint Branch Parkway
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May 12, 2011

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List of Acronyms

ADI	Acceptable daily intake
ANS	Additives and Nutrient Sources
ATCC	American Type Culture Collection
CAS	Chemistry Abstracts Service
CFR	Code of Federal Regulations
CFU	Colony forming unit
CICC	Center of Industrial Culture Collection
EC	European Commission
EDI	Estimated daily intake
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
FAO/WHO	Food and Agriculture Organization/World Health Organization
FCC	Food Chemicals Codex
FDA	United States Food and Drug Administration
FR	Federal Register
GRAS	Generally Recognized as Safe
ITS	Internal Transcribed Spacer
JECFA	Joint Expert Committee on Food Additives
MPN	Most probable number
NOAEL	No observable adverse effect level
NOEL	No observable effect level
SCF	Scientific Committee on Food
UV	Ultra-violet

GRAS EXEMPTION CLAIM

A. Name and Address of Notifier

Baolingbao Biology Co., Ltd. hereby notifies the Food and Drug Administration that the use of erythritol produced via the fermentation of glucose by *Yarrowia lipolytica* (*Y. lipolytica*)[1] described below is exempt from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act because Baolingbao Biology Co., Ltd has determined that such use is generally recognized as safe (GRAS) through scientific procedures.

(b) (6)

May 12, 2011

Echo Q. Bao
Strategic Marketing Manager
Baolingbao Biology Co., Ltd.

Date

B. Name of GRAS Substance

The name of the substance that is the subject of this GRAS determination is erythritol produced via the fermentation of glucose by *Y. lipolytica*. Erythritol ($C_4H_{10}O_4$; CAS No. 149-32-6), or 1,2,3,4-Butanetetrol, is a naturally occurring 4-carbon sugar alcohol.

C. Intended Use and Consumer Exposure

Erythritol produced via the fermentation of glucose by *Y. lipolytica* that is the subject of this GRAS determination is intended for use as a flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant, and texturizer in select categories of food. The maximum proposed uses of erythritol by weight percent in each food category are shown in Table 1 below.

[1] The yeast *Yarrowia lipolytica* was previously classified as *Candida lipolytica*.

Table 1. Proposed Uses of Erythritol (% by weight)

Food Category	Maximum Level of Use
• Reduced- and low-calorie carbonated and noncarbonated beverages • Dairy drinks (chocolate and flavored milks)	3.5%
• Frozen dairy desserts (regular ice cream, soft serve, sorbet) • Puddings (instants, phosphate set) • Yogurt (regular and frozen)	10%
• Bakery fillings (fruit, custard, cream, pudding) • Cakes and cookies (regular and dietetic)	15%
• Fat-based cream used in modified fat/calorie cookies, cakes and pastries • Chewing gum • Soft candies (non-chocolate, plain chocolate, chocolate coated)	60%
• Hard candies (including pressed candy, mints, and cough drops)	99%
• Sugar substitutes (carrier)	100%

D. Basis for GRAS Determination

Baolingbao Biology Co., Ltd.'s GRAS determination for the intended uses of erythritol produced via the fermentation of glucose by *Y. lipolytica* is based on scientific procedures as described under 21 CFR§ 170.30(b).

The intended uses of erythritol produced via the fermentation of glucose by *Y. lipolytica* have been determined to be safe, and have also been determined to be GRAS by demonstrating that safety of intake under the proposed conditions of use is based on knowledge and information that is both publicly available and widely accepted by experts qualified by scientific training and experience to evaluate the safety of substances added to food.

The use of erythritol produced by fermenting glucose using *Moniliella pollinis* (*M. pollinis*) and *Trichosporonoides megachiliensis* (*T. megachiliensis*) in select categories of food was previously determined to be GRAS by Cerestar Holding Co. B.V. and Mitsubishi Chemical Corp., respectively. FDA was notified of the GRAS determinations in 2001 and 2006 (filed as GRN 76 and GRN 208, respectively) and in response to each notification, FDA responded that they had no questions regarding the GRAS determination. As part of these GRAS determinations, the *per user* mean and 90th percentile intakes of erythritol from all intended uses were estimated to be 13 and 30 g/day, respectively. The intended uses of erythritol produced from *Y. lipolytica* are identical to the existing GRAS uses of erythritol produced from *M. pollinis* or *T. megachiliensis* as shown in Table 1 above. The functions of erythritol produced from *M. pollinis* or *T. megachiliensis* in food are identical to the functions of erythritol produced via the fermentation of glucose by *Y. lipolytica*. Erythritol produced via the fermentation of glucose by *Y. lipolytica* therefore provides an alternate organism for the production of erythritol to be used for existing GRAS uses in the food supply.

The safety of erythritol produced from the source organism *Y. lipolytica* for use as an ingredient in food was determined both from an evaluation of the safety of *Y. lipolytica* used to produce the erythritol, and an evaluation of the safety of erythritol itself under the intended conditions of use. The key findings from this evaluation are as follows:

- *Y. lipolytica* is an organism with a history of use for the production of food ingredients. For example, FDA has approved *Y. lipolytica* for use in the production of citric acid. FDA recently indicated that the agency had no concerns regarding the GRAS determination of the use of modified *Y. lipolytica* for use in the production of eicosapentaenoic acid (EPA)-containing oil for use in food. The organism also is used in the production of food flavors. The organism is non-pathogenic in the generally healthy population, though recently the organism has been identified as an emerging opportunistic pathogen in compromised individuals with vascular catheters. However, no reports implicating *Y. lipolytica* as a pathogen in healthy individuals were identified in searches of the literature, and no evidence of toxicogenic or pathogenic potential from the use of *Y. lipolytica* in the production of food ingredients was identified in the literature.
- The microorganism used to produce the erythritol is *Y. lipolytica*, strain CGMCC No. 1431. The specific strain of *Y. lipolytica* used in the production of erythritol is maintained using methods suitable for maintenance of yeasts used in the production of food ingredients. Following the fermentation step in the production of erythritol from *Y. lipolytica*, the fermentation solution is filtered through a membrane to remove microbial cells. The final product therefore does not contain any *Y. lipolytica*.
- Erythritol produced from *Y. lipolytica* is a minimum of 99.5% pure erythritol. The erythritol produced from *Y. lipolytica* complies with specifications for purity and identity of erythritol set forth in the Food Chemicals Codex, Sixth Edition, as does erythritol produced by *M. pollinis* or *T. megachiliensis*. A chromatographic comparison of erythritol produced from *Y. lipolytica* with an erythritol standard indicates that the two products are chemically substantially equivalent. Consequently, food grade erythritol produced from glucose by fermentation with *Y. lipolytica* is indistinguishable from other GRAS food grade erythritol products, namely erythritol produced from glucose by fermentation with either *M. pollinis* or *T. megachiliensis*.
- Using widely known and accepted guidelines developed to evaluate the safety of microbial enzyme preparations for use in food processing, *Y. lipolytica* was determined to be appropriate for use in the production of erythritol intended for use as a component in food.
- The safety of erythritol itself for use as an ingredient in food has been extensively reviewed and evaluated. The previous GRAS determinations of the use of erythritol in foods as noted above were based on published results of acute, subacute, and chronic oral toxicity studies in rats, mice, and dogs and studies conducted to assess the potential carcinogenicity, reproductive and developmental effects, genotoxicity, metabolic fate, and human tolerance.
- The previous GRAS determinations made by Expert Panels were supported by safety evaluations of erythritol conducted by the WHO/FAO Joint Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF) of the European Union. JECFA evaluated erythritol produced from glucose or sucrose by fermentation with either *M. pollinis* or *T. megachiliensis* and assigned it an Acceptable Daily Intake (ADI) "not specified" for use as a sweetening agent. An ADI "not specified" is applicable to a food

substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. In 2003, the SCF reviewed available data on erythritol and concluded that the use of erythritol as a food additive is acceptable. Consistent with the Committee's earlier opinion on other polyols, a numerical ADI for erythritol was not established.

- Findings from a review of the recent published literature continue to support the previous assessment of the safety of these uses of erythritol.

The intended uses of erythritol produced via the fermentation of glucose by *Y. lipolytica* were determined to be safe and GRAS based on the chemical equivalency of the erythritol produced via the fermentation of glucose by *Y. lipolytica* and the erythritol produced by other organisms, the nontoxicogenicity and nonpathogenicity of the *Y. lipolytica* source organism, and on the demonstrated safety of erythritol in animals and in humans.

Determination of the safety and GRAS status of erythritol produced via the fermentation of glucose by *Y. lipolytica* for the direct addition to food under its intended conditions of use was made through the deliberation of an Expert Panel consisting of Joseph F. Borzelleca, PhD, and Michael W. Pariza, PhD, who reviewed a dossier prepared by Exponent as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. They individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of erythritol produced via the fermentation of glucose by *Y. lipolytica*, and unanimously concluded that the uses in select foods of the proposed erythritol product, produced consistent with cGMP and meeting appropriate food-grade specifications described above, are safe. It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data and information would reach the same conclusion. Therefore, erythritol produced via the fermentation of glucose by *Y. lipolytica* is GRAS by scientific procedures under the conditions of use described.

E. Availability of Information

The data and information that serve as the basis for this GRAS determination, as well as the information that has become available since the GRAS determination, will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times from Exponent, 1150 Connecticut Ave, NW, Suite 1100, Washington, DC 20036, telephone 202-772-4953; and email: mmurphy@exponent.com.

Description of Substance

Identity

The subject of this GRAS determination is erythritol produced via the fermentation of glucose by *Yarrowia lipolytica*. The yeast was previously categorized as *Candida lipolytica* and continues to be referred to this name in some literature.

Chemical and Common Names

The chemical name for erythritol is 1,2,3,4-Butanetetrol, and common names include meso-erythritol, tetrahydroxybutane, and erythrite.

Chemical Abstracts Service (CAS) Registry Number

The CAS Registry Number for erythritol (meso-erythritol) is 149-32-6.

Molecular Weight and Empirical and Structural Formulas

The molecular weight of erythritol is 122.12, and its empirical formula is $C_4H_{10}O_4$. The structural formula of erythritol is shown in Figure 1.

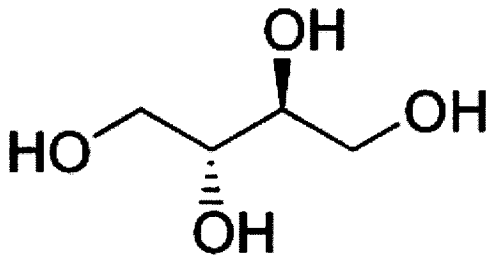

Figure 1. Structural Formula of Erythritol

Production Process

Erythritol is produced through fermentation of glucose catalyzed by the yeast *Y. lipolytica*. Following the fermentation, the resulting erythritol is purified through a series of filtration, concentration, and crystallization procedures. The production process of erythritol is described below, and a detailed production process flow chart is provided in Appendix A.

Fermentation

The fermentation step begins with sterilization of the fermentation tank by steam. Glucose, yeast extract, KH_2PO_4, a defoamer, magnesium sulfate, and ammonium citrate (aqueous ammonia + citrate) are transferred to the fermentation tank via continuous sterilization equipment and *Y. lipolytica* is transferred into the tank under aseptic conditions. Conversion of glucose to erythritol by *Y. lipolytica* occurs through an aerobic fermentation process. Throughout the fermentation, the temperature in the tank is maintained between 25 and 37°C.

Purification

Following the fermentation step, the fermentation solution is filtered through a membrane to completely remove microbial cells. The microorganisms captured in the membrane filtration are killed in a heat treatment step (80°C for 30 minutes). The resulting clear solution from the filtration step is concentrated under reduced pressure to a concentration of 40-60%. Through cooling the solution, the erythritol is crystallized. The erythritol crystals are then separated from the crystal-containing slurry via centrifugation. Activated carbon is added to the redissolved solution for decolorization, and the solution is demineralized using ion-exchange resins. The resulting liquid is then concentrated via a second concentration step followed by vacuum concentration and recrystallization. Erythritol crystals are separated from the crystal-containing slurry via centrifugation and solid erythritol is obtained. A vibrating fluidized bed dryer is then used to dry the crystals, and a mesh screen is used to obtain crystals of the desired particle size. A magnetic separator is employed to ensure that no metal fragments are present in the erythritol crystals. The final erythritol product is packaged in bags.

Product specifications for ingredients and components used in the production of the erythritol were provided in Chinese and translated to English; certification of the translation is provided in Appendix B. Product specifications for ingredients and components used in the production of the erythritol, both in Chinese and English, are shown in Appendix C. All materials used are documented as approved for food use; this documentation is provided in Appendix D.

Product Specifications

The product specifications for erythritol produced via the fermentation of glucose by *Y. lipolytica* are shown in Table 2.

Table 2. Erythritol Specifications

Parameter	Value
Sensory	
Appearance	White crystalline powder
Odor	Odorless
Taste	Sweet and produces cooling sensation upon consumption
Physical and Chemical	
pH	5.0 – 7.0
Melting point	119 – 123°C
Assay	99.5 – 100.5%
Loss on drying	Max 0.2%
Lead	Max 0.1 ppm
Reducing sugars	Max 0.3%
Residue on ignition	Max 0.1%
Ribitol and glycerol	Max 0.1%
Microbiological	
Count of bacteria	Max 500 cfu/g
Yeast and mold	Max 50 cfu/g
Coliforms	Max 10 MPN/g
E. coli	Max 3 MPN/g

MPN = most probable number

Product characterization data from five representative, non-consecutive production lots of erythritol produced via the fermentation of glucose by *Y. lipolytica* are shown in Appendix E. All of the tested parameters were consistent with the product specifications. The methods of analysis also are summarized in Appendix E.

The Food Chemicals Codex, Sixth Edition (FCC 6, U.S. Pharmacopeia 2008) includes a monograph for erythritol obtained from the fermentation broth of the yeast *Moniliella pollinis* or *Trichosporonoides megachiliensis*. The FFC 6 erythritol monograph includes acceptance criteria for the erythritol assay, loss on drying, lead, reducing sugars (as glucose), residue on ignition, and ribitol and glycerol parameters to ensure the purity and identity of erythritol; with the exception of lead, the FCC 6 acceptance criteria for these parameters are identical to the specifications identified in Table 2. As shown in Table 2, the lead specification for erythritol manufactured by Baolingbao Biology Co., Ltd. is 0.1 parts per million (ppm). This limit is 10-fold lower than that specified in the Food Chemicals Codex (1 ppm). The FCC 6 monograph for erythritol, which includes methods of analysis for each parameter, is provided in Appendix F.

Comparison with Erythritol Standard

Based on the assay detailed in the FFC 6 erythritol monograph, Baolingbao Biology Co., Ltd. conducted a high performance liquid chromatography analysis of erythritol produced via the fermentation of glucose by *Y. lipolytica* and also an erythritol standard as defined in the monograph. Results of the comparison with the reference standard indicate that the erythritol produced via the fermentation of glucose by *Y. lipolytica* is chemically substantially equivalent to the standard. The complete report is provided in Appendix G. The report was originally written in Chinese and subsequently translated to English; certification of the translation is provided in Appendix B.

Production Microorganism: *Yarrowia lipolytica*

Identity of the Production Microorganism

The microorganism used to produce the erythritol is *Yarrowia lipolytica*, strain CGMCC No. 1431.[2] This strain of *Y. lipolytica* was obtained from *Y. lipolytica* strain No. 1675 of the China Center of Industrial Culture Collection (CICC), originally obtained from a vegetable source in China, following a combination of common physical and chemical methods to induce mutations resulting in high yield production of erythritol. Specifically, *Y. lipolytica* CICC No. 1675 was exposed to 15w ultra-violet (UV) light for approximately 20-40 minutes and also treated with approximately 100-200 ppm nitrosoguanidine for 30-40 minutes; both of these treatments occurred at room temperature. The rapidly growing mutant strains were then isolated from high osmotic pressure culture medium containing 30% xylitol and 5% glucose and the selected strains were used to ferment in a culture medium containing 40% glucose as the primary carbon source. Selection of the high yield mutant strain of *Y. lipolytica* was made based on production of erythritol from the glucose solution. The selected strain was designated *Y. lipolytica* CGMCC No. 1431. This strain was deposited in the China General Microbiological Culture Collection Center located within The Institute of Microbiology, Chinese Academy of Sciences in Bejing, China.

Strain identification testing on the isolated strain *Y. lipolytica* CGMCC No. 1431 has been completed. Morphological characterization of the organism indicates that the culture is pure; colonies are cream colored, and butyrous to membranous. Vegetative cells are ellipsoidal to elongate and multilateral budding. Pseudohyphae and true hyphae are present. Results from a comparison of the internal transcribed spacer (ITS) region sequence of strain CGMCC No. 1431 indicate that it is identical to the sequence of strain *Y. lipolytica* ATCC Number 9773. ATCC Number 9773 is identified as *Yarrowia lipolytica* (Wickerham et al.) van der Walt et von Arx, teleomorph deposited as *Mycoderma lipolytica*. The alternate state of the organism is identified as *Y. lipolytica* (Harrison) Diddens et Lodder, anamorph. The identification report form of *Y. lipolytica*, strain CGMCC No. 1431, is shown in Appendix H. The report was originally written in Chinese and subsequently translated to English; certification of the translation is provided in Appendix B. ATCC's description of ATCC Number 9773 is also included in Appendix H.

Maintenance of the Production Organism

Baolingbao Biology Co., Ltd. obtained *Yarrowia lipolytica* CGMCC No. 1431 from a supplier for use in production of erythritol. The strain has been maintained at Baolingbao Biology Co., Ltd. since 2003 via a subculture method. Culturing components include glucose, yeast extract, and agar powder. The agar slant medium is inoculated and the culture is stored at a temperature of 4 to 6°C. One subculture is performed each 3 to 6 months. Identification of the strain is based on characteristics including a maximum colony size of approximately 1 to 2 mm, and an initially smooth colony surface, though the appearance is rugose after extended culture. When

[2] The yeast was identified in the China Center of Industrial Culture Collection (the source of the yeast) as *Candida lipolytica,* the original classification of *Yarrowia lipolytica.*

grown in a liquid or solid medium, the yeast cells are spheroidal or ellipsoidal, or alternatively, they may be elongated like pseudohyphal growth with a dimension of approximately 4 to 6 μm x 6 to 8 μm.

Safety of *Yarrowia lipolytica* Used to Produce Erythritol

A comprehensive search of the publicly available literature for information pertaining to the safety of *Y. lipolytica* for use in the production of erythritol was conducted. Databases that were searched include PubMed and Toxline; searches were conducted through March 2011. The information search also included review of reference texts and on-line reference sources. The identified information is reviewed below.

Characteristics of *Yarrowia lipolytica*

The yeast *Yarrowia lipolytica* was originally classified as *Candida* since no sexual state had been described (Barth and Gaillardin 1997). Following the identification of the perfect form (sexual stage), it was reclassified first as *Endomycopsis lipolytica*, then as *Saccharomycopsis lipolytica* and finally as *Yarrowia lipolytica* (Barth and Gaillardin 1997). The species name "*Yarrowia lipolytica*" refers to the extensive capacity this fungus has to degrade n-paraffins and oils (Bankar et al. 2009). The organism is referred to as both *Yarrowia lipolytica* and *Candida lipolytica* in the literature.

Y. lipolytica is a hemiascomycetous yeast in the family Dipodascaceae (Fickers et al. 2005) in the order Saccharomycetales (Bankar et al. 2009). *Y. lipolytica* is the only known taxon in the teleomorph genus *Yarrowia* and has its anamorph in the genus *Candida* as *Candida lipolytica* (F. C. Harrison) Diddens & Lodder (Kurtzman and Fell 1998, as cited in Knutsen et al. 2007). *Y. lipolytica* is an obligate aerobe (Fickers et al. 2005) with a maximum growth temperature in the range of 33 to 37°C (Hazen and Howell 2007). It is heterothallic and dimorphic, as depending on growing conditions, *Y. lipolytica* can form either yeast cells or hyphae and pseudohyphae (Madzak et al. 2004). Carbon sources for the yeast include glucose, alcohols, acetate, and hydrophobic substances including fatty acids and alkanes; *Y. lipolytica* does not use sucrose as a carbon source (Barth and Gaillardin 1997).

Analysis of the genome sequence of *Y. lipolytica* revealed that it is comprised of approximately 20.5 Mb DNA organized in 6 chromosomes (Dujon et al. 2004). *Y. lipolytica* is very distantly related to other yeasts, and instead shares a number of common properties with filamentous fungi (Dujon et al. 2004).

History of Use

Y. lipolytica is isolated from foods (Barth and Gaillardin 1997) and natural environments (Bankar et al. 2009). It also has been found in the oral cavity (Bremenkamp et al. 2010) and on hand skin (McNeil et al. 1999).

Y. lipolytica has a history of consumption due to its presence in select commonly consumed foods. Food products in which the organism has been found typically include products with a high concentration of fat and protein such as dairy products, shoyu (soy sauce), poultry products, sausage, and salads containing meat or shrimp (Barth and Gaillardin 1997; Fickers et al. 2005; Ismail et al. 2000; Jacques and Casaregola 2008; Kumura et al. 2004). Among dairy products, cheese may be a particularly concentrated source of *Y. lipolytica*, as the concentration of the organism frequently exceeds 10^6-10^7 cfu/g in Camembert and blue-veined cheeses (Fickers et al. 2005).

There was considerable interest in *Y. lipolytica* in the mid-1960's based on the organism's ability to use n-paraffins as a sole source of carbon (Bankar et al. 2009; Barth and Gaillardin 1997). Since that time, additional research has been conducted on wild-type and mutant strains, as well as genetically manipulated strains of the yeast for a variety of functions (Bankar et al. 2009). In addition to use of the organism in basic research, applications of the organism include production of aromas such as γ-decalactone, intermediate metabolite production (e.g., citric acid and 2-ketoglutaric acid), single-cell oil (SCO) production, production of lipid-derived compounds such as wax esters, isoprenoid-derived compounds and free hydroxylated fatty acids, in bioremediation processes (Barth and Gaillardin 1997; Fickers et al. 2005, Sabirova et al. 2011), and in the ripening of cheese (Ferreira and Viljoen 2003).

Y. lipolytica is permitted as a secondary direct food additive for fermentation production of citric acid that may be used in food for human consumption (21 CFR 173.165). Recently use of eicosapentaenoic acid (EPA)-containing oil derived from modified *Y. lipolytica* in certain foods was determined to be GRAS based on scientific procedures, provided the combined total intake of EPA and DHA does not exceed 3 g/person/day. Du Pont Applied BioSciences submitted a GRAS notice to FDA summarizing the intended food uses of the oil and the basis for its GRAS determination (du Pont Applied Biosciences 2010). The FDA responded to the submission that the agency had no questions about DuPont's GRAS determination (US FDA 2011).

Safety Data

Y. lipolytica is described as a non-pathogenic organism in 21 CFR 173.165, the regulation permitting its use in the fermentation production of citric acid for human consumption. *Y. lipolytica* ATCC 9773, the strain to which the ITS region sequence of *Y. lipolytica* CGMCC No. 1431 was compared and found to be identical is assigned a biosafety level of 1, indicating that it is not known to cause disease in healthy adult humans (ATCC 2010).

Results from testing in animals also have indicated that *Y. lipolytica* is not pathogenic. In a study of untreated or cortisone treated mice inoculated with *Y. lipolytica*, there was no evidence of pathogenicity of the yeast based on the absence of recovery of *Y. lipolytica* from tissues including kidney, brain, heart, liver and spleen on day 6 following inoculation (Holzschu et al. 1979). Yoshida and Hashimoto (1986) determined *Y. lipolytica* to be of low pathogenic potential based on examination of liver, kidney, and brain tissue of mice 14 days after inoculation with a suspension containing 10^7 cfu of washed, living cells.

Following these early assessments, researchers recovered *Y. lipolytica* from a total of six patients presenting with persistent fungemia with catheter-associated *Candida* thrombophlebitis, polymicrobial sinusitits, and four cases of tissue colonization (Walsh et al. 1989). The isolate that caused clinical fungemia was preserved and groups of two male CD-1 mice were inoculated with one of five dilutions (0.1 mL of 10^4 to 10^8 CFU solutions) of *Y. lipolytica* via the lateral tail vein; a clinical isolate of *C. albicans* was used as a virulence control. All mice inoculated with *Y. lipolytica* survived the 14 day experimental period. Post-mortem examination of the brain, liver, spleen and kidney of each inoculated mouse revealed two abscesses in the left kidney of a mouse that received the highest inoculum (10^8 CFU). Based on these findings, the investigators concluded that *Y. lipolytica* is a weak pathogen associated most clearly with vascular catheter-related fungemia.

In the more recent literature, *Y. lipolytica* has been identified as an emerging opportunistic pathogen (Hazen and Howell 2007; Lopez-Martinez 2010). Case reports have implicated *Y. lipolytica* as a rare pathogen in compromised individuals, including infants with vascular catheters (Belet et al. 2006), a catheterized 13-year-old boy with acute lymphoblastic leukemia (Ye et al. 2011), a pediatric patient with tubercular meningitis (Agarwal et al. 2008), a patient receiving allogenic bone marrow transplantation (D'Antonio et al. 2002), and a patient with acute myelogenous leukemia (Chang et al. 2001). Shin and colleagues (2000) reported that *Y. lipolytica* infection occurred as a nosocomial cluster in five pediatric patients. *Y. lipolytica* has also been isolated from the oral candidal lesions in HIV-positive patients (Tumbarello et al. 1996), and *Y. lipolytica* was found in combination with *Candida* species in infected nails (Segal et al. 1996). Surveillance of bloodstream infections of *Candida* spp. in the U.S. between 1992 and 2001 indicated that the *Y. lipolytica* strain was rarely involved in infections, as this strain was identified in just 4 out of 3683 (0.1%) tested isolates (Pfaller and Diekman 2004). More recently, Pfaller and colleagues (2010) reported that *Y. lipolytica* accounted for 0.05% of *Candida* isolates collected from patients in 142 different medical centers in 41 countries between 1997 and 2007. *Y. lipolytica* has not been associated with disease in healthy humans (Jacques and Casaregola 2008), and reports implicating *Y. lipolytica* as a pathogen in healthy individuals were not identified in searches of the more recent literature.

Pariza and Johnson (2001) developed guidelines that can be used to evaluate the safety of microbial enzyme preparations for use in food processing, including the safety of metabolites of the production strain. Based on these guidelines, the primary consideration in an evaluation of safety of a production strain is the toxigenic potential of the strain. Pathogenic potential typically is not a concern, as food enzyme preparations rarely contain viable production organisms. Additionally, Pariza and Johnson note that it is important to distinguish between pathogenicity and opportunistic infection. When given access to tissue normally protected by the host, many microorganisms will produce opportunistic infections. True pathogens, however, are able to cross uncompromised host barriers and produce infections in individuals that are generally regarded as healthy.

An analysis of erythritol produced by *Y. lipolytica* CGMCC No. 1431, using the Pariza-Johnson (2001) decision tree, is presented in Appendix I. It is concluded that *Y. lipolytica* CGMCC No. 1431 is an appropriate production organism for the manufacture of erythritol intended for use as a component in food, because the erythritol product produced using this strain is substantially

equivalent to erythritol products already determined to be GRAS (filed as GRN 76 and GRN 208).

Historical Consumption and Current Regulated Uses

Historical Consumption

Erythritol is a naturally occurring 4-carbon sugar alcohol that has a sweetness of approximately 60 to 80% that of sucrose (Berndt et al. 1996). It is found in commonly consumed fermented foods including cheese, wine, sake, beer, and soy sauce; mushrooms; and some fruits such as watermelon, pears, and grapes (Berndt et al. 1996; Sreenath and Venkatesh 2008). Naturally occurring concentrations of erythritol in foods are as high as approximately 0.13% (w/v) (Berndt et al. 1996). Erythritol also has been found to exist endogenously in tissues and body fluids including plasma and urine of humans and animals (Berndt et al. 1996).

Regulated Uses

Erythritol has a history of use as a food ingredient in many countries around the world. It is used in foods primarily as a low calorie sweetener, though it also provides technical functions in foods including flavor enhancer, formulation aid, humectant, stabilizer and thickener, sequestrant, and texturizer (Berndt et al. 1996).

In the United States

Cerestar Holding Co. B.V., Mitsubishi Chemical Corp., and Nikken Chemicals Co., Ltd., jointly filed a petition (GRASP 7G0422) to the U.S. Food and Drug Administration (FDA) proposing to affirm that the use of erythritol is generally recognized as safe (GRAS) as an ingredient in human food (FR 1997). The GRAS determination was made by a panel of food safety experts (Berndt et al. 1996). The proposed applications of erythritol included use as a flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant and texturizer at maximum levels ranging from 1.5 to 100% in sugar substitutes (100%); hard candies (50%); soft candies (40%); reduced- and low-calorie beverages (1.5%); fat-based cream for use in cookies, cakes, and pastries (60%); dietetic cookies and wafers (7%); and chewing gum (60%) (Berndt et al. 1996). The data and information contained in the petition submitted to FDA were developed jointly by the three submitters and pertained to erythritol produced by fermenting glucose using either *Moniliella pollinis* (*M. pollinis*) or *Trichosporonoides megachiliensis* (*T. megachiliensis*).

In 2000, the same Expert Panel convened and determined that erythritol produced from *M. pollinis* and meeting specifications for erythritol as described in the FCC at that time (FCC 4th Edition, Second Supplement) was GRAS for use in a slightly expanded list of foods and some increased levels of use as compared to uses addressed in GRASP 7G0422, which remained pending with FDA at that time. The intended uses addressed in the 2000 GRAS determination included expanded or new uses in bakery fillings, reduced- and low-calorie beverages, regular cakes and cookies, dairy drinks, frozen dairy desserts, puddings (instant, phosphate set), and yogurt. The Expert Panel estimated the mean and 90th percentile per user intakes of erythritol to

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be 14.1 and 30.8 g/day based on the maximum proposed use of erythritol in all use categories. The Expert Panel then applied a market share adjustment and assumed that erythritol would replace all xylitol use (20% of total polyol use) and reported projected per user mean and 90th percentile intakes of 4.9 and 11.9 g/day, respectively. Additionally, the Expert Panel noted that 90th percentile intakes by young children ages 1 to 3 years may be in the range of 1 g/kg bw/day.

FDA was notified of the Expert Panel's GRAS determination. The notification was filed as GRN 76 (Cerestar 2001). FDA responded that they had no questions regarding Cerestar's conclusion of general recognition of safety (FDA 2001). In the response letter, FDA noted that by their own calculations, estimated intake of erythritol under the proposed conditions of use would result in *per user* intakes of 13 g/day at the mean, and 30 g/day at the 90th percentile (FDA 2001). In 2006, the Mitsubushi-Kagaku-Foods Corporation notified FDA that erythritol produced from *T. megachiliensis* and meeting specifications for erythritol as described in the FCC at that time (FCC 5th Edition) was GRAS for use under the same expanded conditions specified in Cerestar's notification (Mitsubushi-Kagaku-Foods Corporation 2006). The notification was filed as GRN 208. FDA responded to the Mitsubushi-Kagaku-Foods Corporation that they had no questions regarding the GRAS determination (FDA 2007).

In the United States, erythritol is included among the sugar alcohols that may be the subject of an authorized health claim on sugar alcohols and dental caries (21 CFR 101.80).

Outside the United States

Erythritol has been used in Japan since 1990 as a component of candies, sugar substitutes, chocolates, soft drinks, chewing gum, jellies, jams, and yogurt (Berndt et al. 1996). Erythritol is also permitted for use in foods and in beverages in other countries around the world, including China, Russia, Canada, Australia, Hong Kong, New Zealand, Brazil, Argentina, Uruguay, Mexico, Korea, Thailand, Singapore, Taiwan, Hong Kong, Philippines, Israel, and South Africa (EFSA 2010).

At its fifty-third meeting, the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) evaluated erythritol produced from glucose or sucrose by fermentation with either *M. pollinis* or *T. megachiliensis* and assigned it an Acceptable Daily Intake "not specified" for use as a sweetening agent (JECFA 2000). JECFA established specifications for erythritol obtained by fermentation of starch enzyme hydrolysate by safe and suitable osmophilic yeasts such as *M. pollinis* or *T. megachiliensis* (JECFA 2000). As previously noted, the Food Chemicals Codex also has developed standards for the purity and identity of erythritol obtained from the fermentation broth of the yeast *M. pollinis* or *T. megachiliensis* (U.S. Pharmacopeia 2008).

In 2003, the Scientific Committee on Food (SCF) of the European Commission (EC) released their opinion in which they concluded that use of erythritol as a food additive is acceptable, and, consistent with earlier opinions on polyols, stated that it was inappropriate to establish a numerical ADI for erythritol (SCF 2003). In the European Union, erythritol may be used for

sweetening purposes like the other permitted polyols, and may be added to foods for purposes other than sweetening at quantum status levels as specified in Directive 2006/52/EC (EC 2006).

Erythritol produced via the fermentation of glucose by *Y. lipolytica* (produced by Baolingbao Biology Co., Ltd.) has been available since 2003. Between 2003 and 2010, over 12 MT of the product have been produced and distributed in China, Japan, Korea and Norway.

Intended Use and Estimated Intakes

Intended Use

The intended uses of erythritol produced from *Y. lipolytica* are identical to the existing GRAS uses of erythritol produced from *M. pollinis* or *T. megachiliensis* (Cerestar 2001; Mitsubushi-Kagaku-Foods Corporation 2006); these uses are listed in Table 3. The functions of erythritol in food include flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant, and texturizer.

Table 3. Intended Uses of Erythritol

Food Category	Maximum Level of Use
• Reduced- and low-calorie carbonated and noncarbonated beverages • Dairy drinks (chocolate and flavored milks)	3.5%
• Frozen dairy desserts (regular ice cream, soft serve, sorbet) • Puddings (instants, phosphate set) • Yogurt (regular and frozen)	10%
• Bakery fillings (fruit, custard, cream, pudding) • Cakes and cookies (regular and dietetic)	15%
• Fat-based cream used in modified fat/calorie cookies, cakes and pastries • Chewing gum • Soft candies (non-chocolate, plain chocolate, chocolate coated)	60%
• Hard candies (including pressed candy, mints, and cough drops)	99%
• Sugar substitutes (carrier)	100%

Estimated Daily Intakes

As noted previously, the estimated daily intake (EDI) *per user* of all intended uses of erythritol previously was calculated by FDA to be 13 g/day at the mean and 30 g/day at the 90th percentile (US FDA 2001). These intakes are comparable to the *per user* mean and 90th percentile estimates calculated by Cerestar for the expanded uses (14.1 and 30.8 g/day, respectively). Given that the intended uses of erythritol are identical across of sources of the sugar alcohol (i.e., produced by fermenting glucose using either *Y. lipolytica*, *M. pollinis*, or *T. megachiliensis*), the previously calculated estimates also provide valid estimates of intake of erythritol produced from *Y. lipolytica*.

Safety Data

Introduction

The safety of erythritol for use as an ingredient in food has been extensively reviewed and evaluated. The GRAS status of erythritol for use in a variety of foods, including uses identical to the uses proposed for erythritol produced from *Y. lipolytica*, was previously established. These safety determinations are briefly reviewed below. Findings from a review of the published literature since the most recent publicly available erythritol safety determination also are summarized.

Safety of Erythritol

Information Used to Establish GRAS Status of Erythritol from *M. pollinis* and *T. megachiliensis*

The safety of erythritol for use as an ingredient in food has been extensively reviewed and evaluated. As previously noted, the GRAS status of erythritol for use in a variety of foods was established by a panel of qualified food safety experts (Berndt et al. 1996), and subsequently GRAS status was established for expanded uses in foods (Cerestar 2001; Mitsubushi-Kagaku-Foods Corporation 2006). The expanded GRAS applications of erythritol include uses up to the specified maximum levels in the following categories of foods: reduced- and low calorie carbonated and non-carbonated beverages (3.5%); dairy drinks (chocolate and flavored milks) (3.5%); frozen dairy desserts (regular ice cream, soft serve, sorbet) (10%); puddings (instant, phosphate set) (10%); yogurt (regular and frozen) (10%); bakery fillings (fruit, custard, cream, pudding) (15%); cakes and cookies (regular and dietetic) (15%); fat-based cream used in modified fat/calorie cookies, cakes and pastries (60%); chewing gum (60%); soft candies (non-chocolate, plain chocolate, chocolate coated) (60%); hard candies (including pressed candy, mints and cough drops) (99%); and sugar substitutes (carrier) (100%).

The general recognition of safety for these uses of erythritol in foods was based on a critical review of data and information in the published literature. A comprehensive, critical review and summary of the biochemical, metabolic, toxicological, and clinical data was published in 1998 (Munro et al. 1998). As detailed in the published review (Munro et al. 1998) and summarized by Cerestar's GRAS Expert Panel (Cerestar 2001), the safety of erythritol for use in foods was established based on results of acute, subacute, and chronic oral toxicity studies in rats, mice, and dogs. Studies also were conducted to assess the carcinogenic potential of erythritol, teratogenicity, reproductive effects, genotoxicity, metabolic fate, and human tolerance.

Comprehensive safety reviews of erythritol also were conducted by Joint FAO/WHO Expert Committee on Food Additives (JECFA 2000) and the Scientific Committee on Food (SCF) of the European Union (SCF 2003). Summaries of these publicly available reviews were included in the GRAS notifications submitted to FDA for erythritol.

Key findings from the published review (Munro et al. 1998) and the notifications to FDA (Cerestar 2001, Mitsubushi-Kagaku-Foods Corporation 2006), the JECFA review and the EFSA review are summarized below.

Munro et al. 1998 and GRAS Notifications to FDA (Cerestar 2001, Mitsubushi-Kagaku-Foods Corporation 2006)

Results of studies in both animals and humans demonstrated that erythritol is quickly absorbed, is not metabolized systemically, and is excreted unchanged in the urine. Based on studies in humans, approximately 90% of the ingested erythritol is absorbed, leaving up to 10% of the ingested erythritol potentially available for fermentation by the colonic microflora. Results from *in vitro* incubation studies, including a more recent study (Arrigoni et al. 2005), suggest that colonic fermentation of erythritol in humans is unlikely. Because the absorbed portion of erythritol is not systemically metabolized, it does not contribute to the caloric load.

No adverse toxicological effects were observed in studies in which animals consumed up to 20% erythritol in the diet. At high doses, erythritol treatment of animals was associated with some physiological changes including transient diarrhea, increased cecal and kidney weights, increased water consumption and urine output, and minor changes in some urinary parameters. No histopathological evidence of toxicity was observed, however, and the effects reversed after cessation of exposure. The observed effects were considered to be physiological responses to high loads of poorly absorbed low molecular weight compounds and of no toxicological significance. Additionally, there was no evidence that erythritol has any carcinogenic, mutagenic, or teratogenic potential, and no effects on reproductive performance or fertility were reported.

In clinical studies of erythritol, some minor, transient gastrointestinal effects were observed, though only after intake of a large acute bolus (liquid) dose and/or intake on an empty stomach. A laxation threshold for erythritol was not clearly identified in the clinical studies. The available data suggested, however, that it may be in excess of 0.6 to 0.8 g erythritol/kg bw under all conditions of use, or approximately 1 g erythritol/kg bw/day or more in divided doses in foods and beverages. Intakes of up to 1 g erythritol/kg bw/day in divided doses also were not associated with meaningful renal effects. An intake of 1 g erythritol/kg bw/day corresponds to a daily intake of 60 g erythritol for a 60 kg adult.

JECFA 2000

JECFA evaluated erythritol produced from glucose or sucrose by fermentation with either *Moniliella pollinis* or *Trichosporonoides megachiliensis* and assigned it an Acceptable Daily Intake "not specified" for use as a sweetening agent (JECFA 2000). An ADI "not specified" is applicable to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological, and other), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. For that reason, and for reasons stated in individual evaluations, the establishment of an acceptable daily intake expressed in numerical form is not deemed necessary. JECFA's specification for

erythritol states that the sugar alcohol may be produced via fermentation of starch enzyme hydrolysate "by safe and suitable food grade osmophilic yeasts such as *Moniliella pollinis* or *Trichosporonoides megachilensis*" (JECFA 1999).

SCF 2003

In 2003, the SCF reviewed available data on erythritol and concluded that the use of erythritol as a food additive is acceptable. The Committee cited rare reports of allergic and allergic-like adverse reactions after ingestion of erythritol. The Committee determined the no observable effect level (NOEL) for the laxative effect of erythritol to be approximately 0.5 g erythritol/kg bw when administered as a single dose in solution on an empty stomach; this is equivalent to a bolus intake of approximately 30 g erythritol for an adult weighing 60 kg. Consistent with the Committee's earlier opinion on other polyols, it was determined inappropriate to establish a numerical ADI for erythritol.

Erythritol Safety Data Published Since 2006

A search of PubMed for information related to the safety of erythritol published since 2006, the year of the most recent Notification to FDA of the GRAS status of erythritol under its intended conditions of use, was conducted through March 2011 to determine if more recent information continues to support the previously determined GRAS status.

Two studies of the effects of varying bolus doses of erythritol were identified in the search (Oku and Nakamura 2007, Story et al. 2007). Results from both of these studies continue to support the safe intake of erythritol under the GRAS conditions of use.

Storey and colleagues (2007) examined gastrointestinal tolerance of single, oral, bolus doses of 20, 35, or 50 g of an aqueous solution of erythritol as compared to comparable bolus doses of xylitol or a 45 g bolus dose of sucrose in a population of 64 healthy adults age 18-24 years in the United Kingdom. The test beverages were consumed within 15 minutes either as a mid-morning or mid-afternoon beverage after having consumed a normal breakfast or lunch. Doses of 20 or 35 g erythritol in water were well tolerated by the study volunteers. Doses of 50 g erythritol were associated with significant increases in the number of subjects reporting nausea or borborygmi as compared to effects after consuming sucrose in water. The 50 g dose of erythritol had no significant effect on gastrointestinal symptoms including bloating, colic, flatulence, or watery feces. The absence of any significant increase in the number of subjects with watery feces suggests that a 50 g dose of erythritol, the highest dose tested (corresponding to approximately 0.78 g erythritol/kg bw), was below the threshold of laxation.

Oku and Nakamura (2007) examined the threshold of transitory diarrhea following intake of 20, 30, 40, or 50 g erythritol in water by 55 young healthy adults (27 males, mean age 19.9 years; 28 females, mean age 21.2 years) in Japan as compared to doses of xylitol or lactitol. Responses to doses of 10, 20, 30, 40, and 50 g xylitol and 10, 20, 30 and 40 g lactitol (each provided in aqueous solutions) also were assessed in the study. The beverages were consumed two to three hours following a meal, usually breakfast. Increasing doses of the test substances were

consumed until diarrhea was reported. Intake of 20 or 30 g erythritol was well tolerated by men and women, with no reported cases of diarrhea. One quarter of the men experienced diarrhea following intake of 40 g erythritol, and the cumulative proportion of men experiencing diarrhea after intake of 50 g erythritol was 46%. Intake of a bolus dose of 40 g erythritol resulted in diarrhea in approximately 8% of the 26 tested women, while 31% of the women experienced diarrhea following intake of 50 g erythritol. The laxative threshold of erythritol was calculated to be 0.46 g erythritol/kg bw for males, and 0.68 g erythritol/kg bw for females. The investigators noted that these values were slightly lower than thresholds they previously reported (0.6 and 0.8 g erythritol/kg bw for males and females, respectively) and suggested that the differences may be attributed to one or more differences between the two studies including the test article matrix (water versus jelly), age of the subjects, location of the experiment, and season of the year. As noted above, Storey and colleagues (2007) also examined responses to an aqueous dose of erythritol. In their study in healthy young adults, an aqueous dose of 50 g erythritol, which was equivalent to 0.78 g erythritol/kg bw, was below the laxative threshold. These findings suggest that test article matrix alone may not account for the reduced threshold observed by Oku and Nakamura in their 2007 study.

Recently the Panel on Food Additives and Nutrient Sources (ANS) of the European Food Safety Authority (EFSA) released on opinion on the tolerability of erythritol as consumed in beverages by young children (EFSA 2010). Using a double-blind, randomized, controlled cross-over design, the tolerance of a bolus dose of 5, 15 or 25 g erythritol was assessed in 128 children age 4 to 6 years in France. Bolus doses of 5 or 15 g erythritol (0.25 or 0.71 g/kg bw, respectively) resulted in no significant differences in incidence of diarrhea or meaningful gastrointestinal symptoms as compared to responses after intake of the control product (a saccharose sweetened product). A bolus dose of 25 g erythritol (1.23 g/kg bw), the next highest dose tested in the study, resulted in a significant increase in the incidence of diarrhea and/or significant gastrointestinal symptoms as compared to the control group (32% versus 3.4%; p=0.0002). Based on these findings, a single dose of 15 g erythritol, corresponding to 0.71 g erythritol/kg bw, was determined to be the no observed adverse effect level (NOAEL) for children.

Overall, as summarized by the Expert Panel in the review of erythritol safety (Cerestar 2001), the data available at that time suggested that the threshold of laxation of erythritol is in excess of 0.6 to 0.8 g erythritol/kg bw. As noted in the review by Munro and colleagues (1998), transient adverse gastrointestinal effects following intake of erythritol are most likely seen after intake of a large bolus dose in a liquid form on an empty stomach. Evidence from recent large, well conducted bolus dose studies, including a study in young children, continues to support the previous conclusion that the threshold of laxation of erythritol is in excess of 0.6 to 0.8 g erythritol/kg bw.

The maximum proposed use of erythritol in beverages including reduced- and low-calorie carbonated or noncarbonated beverages and dairy drinks (chocolate and flavored milks) is 3.5%. Assuming young children age 2-6 years consume a 240 mL beverage serving during an eating occasion, the maximum erythritol intake is 8.4 g per occasion, which is equivalent to 0.56 mg/kg bw per occasion assuming a default body weight of 15 kg. Likewise, assuming a person in the U.S. population age 2 years and older consumes two beverage servings during a single eating occasion (480 mL), the maximum erythritol intake is 16.8 g per eating occasion, or 0.28 mg/kg

bw per eating occasion assuming a default body weight of 60 kg. Among subpopulations of children and the total population in the U.S., the hypothetical maximum intake of erythritol per eating occasion from beverages is therefore below the threshold of laxation.

Basis for the GRAS Determination

Introduction

The regulatory framework for determining whether a substance can be considered generally recognized as safe (GRAS) for its intended use in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data information.

These criteria are applied in the analysis below to determine whether erythritol produced via the fermentation of glucose by *Yarrowia lipolytica* is GRAS based upon scientific procedures.

Safety Determination

The subject of this GRAS determination is erythritol produced via the fermentation of glucose by *Yarrowia lipolytica*. The yeast *Y. lipolytica* is also referred to as *Candida lipolytica (C. lipolytica)*. The intended uses of erythritol produced via the fermentation of glucose by *Y. lipolytica* are identical to current GRAS uses of erythritol produced via the fermentation of glucose by two other yeasts, *M. pollinis*, or *T. megachiliensis*. Erythritol produced via the fermentation of glucose by *Y. lipolytica* therefore would provide an alternate organism for the production of erythritol. The safety of erythritol produced from *Y. lipolytica* for use as an ingredient in food can be determined from an evaluation of the safety of *Yarrowia lipolytica* used to produce the erythritol, and an evaluation of the safety of erythritol under the intended conditions of use.

Safety of *Y. lipolytica* to Produce Erythritol

Yarrowia lipolytica is an organism with a history of use for the production of food ingredients, as the FDA has approved *Y. lipolytica* for use in the production of citric acid, FDA recently indicated the agency had no concerns regarding the GRAS determination of the use of modified *Y. lipolytica* for use in the production of EPA-containing oil for use in food, and the organism is used in the production of food flavors. The organism is non-pathogenic in the generally healthy population, though recently the organism has been identified as an emerging opportunistic pathogen in compromised individuals with vascular catheters. No reports implicating *Y. lipolytica* as a pathogen in healthy individuals were identified in searches of the literature. No evidence of toxicogenic or pathogenic potential from the use of *Y. lipolytica* in the production of food ingredients was identified in the literature.

The microorganism used to produce the erythritol is *Y. lipolytica*, strain CGMCC No. 1431. The specific strain of *Y. lipolytica* used in the production of erythritol is maintained using methods suitable for maintenance of yeasts used in the production of food ingredients. Following the fermentation step in the production of erythritol from *Y. lipolytica*, the fermentation solution is filtered through a membrane to remove microbial cells. The final product therefore does not contain any *Y. lipolytica*.

Erythritol produced from *Y. lipolytica* is a minimum of 99.5% pure erythritol. The erythritol produced from *Y. lipolytica* complies with specifications for purity and identity of erythritol set forth in the Food Chemicals Codex, 6th Edition, as does erythritol produced by *M. pollinis* or *T. megachiliensis*. A chromatographic comparison of erythritol produced from *Y. lipolytica* with an erythritol standard indicates that the two products are chemically substantially equivalent. Consequently, food grade erythritol produced from glucose by fermentation with *Y. lipolytica* is indistinguishable from other GRAS food grade erythritol products, namely erythritol produced from glucose by fermentation with either *M. pollinis* or *T. megachiliensis*.

Pariza and Johnson (2001) developed guidelines that can be used to evaluate the safety of microbial enzyme preparations for use in food processing, including the safety of metabolites of the production strain. Based on these guidelines, the primary consideration in an evaluation of safety of a production strain is the toxigenic potential of the strain. Pathogenic potential typically is not a concern, as food enzyme preparations rarely contain viable production organisms. Additionally, Pariza and Johnson note that it is important to distinguish between pathogenicity and opportunistic infection. When given access to tissue normally protected by the host, many microorganisms will produce opportunistic infections. True pathogens, however, are able to cross uncompromised host barriers and produce infections in individuals that are generally regarded as healthy. *Y. lipolytica* therefore is appropriate for use in the production of erythritol intended for use as a component in food.

Safety of Erythritol

The safety of erythritol for use as an ingredient in food has been extensively reviewed and evaluated. The GRAS status of the uses of erythritol in a variety of foods, including uses

identical to the uses proposed for erythritol produced from *Y. lipolytica*, was previously established based on information available in the publicly available literature. These levels of erythritol in the U.S. food supply were first recognized as GRAS by a panel of experts qualified by scientific training and experience in 2000. Erythritol meets food grade specifications as established by the FCC. Maximum uses of erythritol in foods determined to be GRAS are as follows: reduced- and low calorie carbonated and non-carbonated beverages (3.5%); dairy drinks (chocolate and flavored milks) (3.5%); frozen dairy desserts (regular ice cream, soft serve, sorbet) (10%); puddings (instant, phosphate set) (10%); yogurt (regular and frozen) (10%); bakery fillings (fruit, custard, cream, pudding) (15%); cakes and cookies (regular and dietetic) (15%); fat-based cream used in modified fat/calorie cookies, cakes and pastries (60%); chewing gum (60%); soft candies (non-chocolate, plain chocolate, chocolate coated) (60%); hard candies (including pressed candy, mints and cough drops) (99%); and sugar substitutes (carrier) (100%).

Given that the intended uses of erythritol are identical across all sources of the sugar alcohol (i.e., produced by fermenting glucose using *Y. lipolytica*, *M. pollinis*, or *T. megachiliensis*), the previously calculated estimates of erythritol intake also provide valid estimates of potential intake of erythritol produced from *Y. lipolytica*. The estimated *per user* mean intake of all intended uses of erythritol is 13 g/day, and the *per user* 90th percentile intake is 30 g/day.

FDA was notified of the GRAS determinations for these uses of erythritol, first in 2001 and again in 2006, and responded with no concerns regarding the assessments. Findings from a review of the recent published literature continue to support the previous assessment of the safety of these uses of erythritol. The uses of erythritol remain GRAS and the intended uses of erythritol produced via the fermentation of glucose by *Y. lipolytica* are therefore GRAS.

Conclusions

The intended uses of erythritol produced via the fermentation of glucose by *Yarrowia lipolytica* are safe and GRAS based on the chemical equivalency of the erythritol produced via the fermentation of glucose by *Y. lipolytica* and the erythritol produced by other organisms, the nontoxicogenicity and nonpathogenicity of *Y. lipolytica*, and on the demonstrated safety of erythritol in animals and in humans.

General Recognition of the Safety of *Yarrowia lipolytica* to Produce Erythritol

The intended use of erythritol produced via the fermentation of glucose by *Y. lipolytica* has been determined to be safe through scientific procedures as set forth in 21 CFR§170.30(b), thus satisfying the so-called "technical" element of the GRAS determination. Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination. Determination of the safety and GRAS status of erythritol produced via the fermentation of glucose by *Y. lipolytica* for addition to foods under its intended conditions of use has been made through the

deliberations of an Expert Panel comprised of Joseph F. Borzelleca, Ph.D. and Michael W. Pariza, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to food. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that erythritol produced via the fermentation of glucose by *Y. lipolytica*, produced consistent with Good Manufacturing Practice and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further unanimously concludes that these uses of erythritol produced via the fermentation of glucose by *Y. lipolytica* are GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food ingredients would concur with these conclusions. The Panel's GRAS opinion is included as Exhibit I to this document.

Exponent is not aware of information that would be inconsistent with a finding that the proposed use of erythritol produced via the fermentation of glucose by *Y. lipolytica* as meeting appropriate specifications and used according to Good Manufacturing Practice, is GRAS. A recent search of the scientific literature revealed no potential adverse health concerns.

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Appendices

Appendix A. Detailed Production Process Flow Chart

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Detailed Process Flow Chart of Erythritol

Flow chart	Detailed Information
Receiving of Raw Materials	1. Raw materials have to be inspected strictly, and sampling inspection is performed.
Glucose	1. Transfer the glucose to fermentation tank. 2. The glucose solution is heated for sterilization.
Fermentation	1. By fermentation, glucose can be converted to erythritol catalyzed by a microorganism named Candida lipolytica. 2. This is an aerobic fermentation process. 3. In this process, yeast extract, KH_2PO_4, defoamer, magnesium sulfate, and ammonium citrate(aqueous ammonia + citric acid) are added in to fermentation tank.
Membrane Filtration	1. Through this process, microbial cells and solid impurities can be removed effectively. Get clear solution.
Concentration	1. Concentrate under reduced pressure 2. The final concentration is about 40-60%.
First crystallization	Through this process, erythritol is crystallized
First Centrifugation	Separating erythritol crystal from crystal-containing slurry and obtaining solid erythritol.

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Dissolution Decolorization	1. This is a product purification process. 2. Active carbon is added.
Ion-exchange	Demineralize using columns filled with positive or negative exchange resin.
Second Concentration	Concentrating product liquid
Vacuum-Concentration	Concentrating product liquid
Second Crystallization	Obtain the erythritol crystal
Second Centrifugation	Separating erythritol crystal from crystal-containing slurry and obtaining solid erythritol.
Crystal Drying	Vibrated Fluidized Bed Dryer is used for crystal drying.
Distinguish Mesh	Obtain desired particle size of product.
Metal Detecting	Magnetic separator is applied to inspect and remove metal impurity.
Packaging	

Appendix B. Certification of Chinese to English Translations

III

I, Zhe Li, of Global Language Solutions, hereby certify that the foregoing document, to the best of our knowledge and belief, is a true, complete and accurate English language translation of the attached Chinese language document.

1) Active carbon-CN.pdf
2) Anion exchange resin-CN.pdf
3) Aqueous ammonia-CN.pdf
4) BL-8_Endotoxin Chromatogram
5) BL-8 Microscopic identification.pdf
6) Cation exchange resin-CN.pdf
7) Citric Acid-CN.pdf
8) Dextrose-CN.pdf
9) Magnesium sulfate-CN.pdf
10) Potassium dihydrogen phosphate-CN.pdf
11) Yeast extract-CN.pdf

This document was translated by two or more professionals with adequate experience and qualifications in the medical field to properly interpret and translate such documents.

ALISO VIEJO, CALIFORNIA
January 28th, 2011

(b) (6)

Zhe Li
GLOBAL LANGUAGE SOLUTIONS

State of California
County of Orange

On January 28, 2011 before me, Betsy Rodriguez Notary Public, personally appeared Zhe Li, who proved to me on the basis of satisfactory evidence to be the person whose name is subscribed to the within instrument and acknowledged to me that she executed the same in her authorized capacity, and that by her signature on the instrument the person, or the entity upon behalf of which the person acted, executed the instrument.

I certify under PENALTY OF PERJURY under the laws of the State of California that the foregoing paragraph is true and correct.

WITNESS my hand and official seal.

(b) (6)

Signature

7/7/08

F-140.007 AC

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Appendix C. Specifications for Materials Used in the Production of Erythritol

Baolingbao Biology Co., Ltd.

Raw Materials Standards

Document Section #	Material	Reference Standard
6.4	Active Carbon	GB/T 13803.3 -1999
6.22	Macroporous weak alkaline styrene series anion exchange resin	HGT2624-1994 D301-FC
6.35	Aqueous Ammonia	--
6.21	Macroporous acidic styrene series cation exchange resin	GB 16579-1996-T
6.18	Citric acid	GB 1987-2007
6.34	Defoamer	--
6.7	Magnesium Sulfate	QB 2555-2002
6.33	Potassium Dihydrogen Phosphate	--
6.32	Yeast Extract	QB 23530-2009

6.4 活性炭

6.4.1 参考 GB/T 13803.3-1999《糖液脱色用活性炭》（见附件 4）标准。

6.4.2 标准要求

质量项目	判定标准			到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
外观	黑色粉末状			√		√	√
A 法焦糖脱色率，%	≥90			√		√	√
B 法焦糖脱色率，%	≥90					√	√
水分，%	≤10	≤40	≤55	√		√	√
pH 值	2.0～5.0			√		√	√
灰分含量，%	≤4.0			√		√	√
酸溶物含量，%	≤1.50				√	√	√
铁含量，%	≤0.1					√	√
氯化物含量，%	≤0.25				√	√	√
砷(As)含量(以干基计) %	≤0.0003						√
重金属(以 Pb 计)含量(以干基计) %	≤0.002						√
铅(Pb)含量(以干基计) %	≤0.001						√

注：1)A 法焦糖脱色率与 B 法焦糖脱色率任取其一项为脱色率指标。
2)磷酸法生产的活性炭灰分可在 7%-9%。

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、供方每季度提供一次标准中要求全部检测项目的检测报告；
5、第三方检测项目，每半年进行 1 次。

6.4 Active carbon

6.4.1 Refer to GB/T 13803.3-1999 "Active resin for sugar decolorization" (see Attachment 4) standards.

6.4.2 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
Appearance	Black powder	✓		✓	✓
Method A caramel decolorization rate, %	> 90	✓		✓	✓
Method B caramel decolorization rate, %	> 90			✓	✓
Moisture, %	<10 | <40 | <55	✓		✓	✓
pH value	2.0 – 5.0	✓		✓	✓
Ash content, %	< 4.0	✓		✓	✓
Acid soluble content, %	< 1.50		✓	✓	✓
Iron content, %	< 0.1			✓	✓
Chloride content, %	< 0.25		✓	✓	✓
Arsenic (As) content (calculated on dry basis), %	< 0.0003				✓
Heavy metal (calculated as Pb) content (calculated on dry basis), %	< 0.002				✓
Lead (Pb) content (calculated on dry basis), %	< 0.001				✓

Note: 1) Any item can be used as the decolorization rate index for the method A caramel decolorization rate and method B caramel decolorization rate.
2) The ash content of the active carbon produced by the phosphoric acid method can be 7–9%.

Note: 1. In plant inspection, every batch of product must be inspected.
2. In unscheduled sampling, one out of every 5 batches of product must be inspected.
3. An accompanying report must be provided for each batch of product.
4. Suppliers must provide an inspection report for all inspection items required in the standards once per quarter.
5. The third-party inspection must be performed once every 6 months.

6.22　大孔弱碱性苯乙烯系阴离子交换树脂

6.22.1 参考 HGT 2624-1994 D301-FC　《大孔弱碱性苯乙烯系阴离子交换树脂》（见附件 22）。

6.22.2 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
外观	乳白色或浅黄色不透明球状颗粒	√		√	√
含水量，%	50～60		√	√	√
质量全交换容量，mmol/g	≥4.8		√	√	√
体积全交换容量，mmol/ml	≥1.4		√	√	√
湿视密度，g/ml	0.65～0.72			√	√
湿真密度，g/ml	1.03～1.07			√	√
粒度%（粒径mm）	≥95（0.45～1.25）			√	√
下限粒度%（粒径mm）	≤1（≤0.45）				√
有效粒径，mm	0.50～0.75			√	√
均一系数	≤1.60			√	√
渗磨圆球率，%	≥90			√	√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、第三方检测项目，每批进行 1 次。

6.22 Macroporous weak alkaline styrene series anion exchange resin

6.22.1 Refer to HGT 2624-1994 D301-FC "Macroporous weak alkaline styrene series anion exchange resin" (see Attachment 22).

6.22.2 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
Appearance	Milky white or light yellow opaque spherical pellets	✓		✓	✓
Moisture, %	50 – 60		✓	✓	✓
Quality Full exchange capacity, mmol/g	> 4.8		✓	✓	✓
Volume Full exchange capacity, mmol/ml	> 1.4		✓	✓	✓
Wet superficial density, g/ml	0.65 – 0.72			✓	✓
Wet real density, g/ml	1.03 – 1.07			✓	✓
Granularity, % (grain size, mm)	> 95 (0.45 – 1.25)			✓	✓
Lower-limit granularity, % (grain size, mm)	< 1 (< 0.45)				✓
Effective grain size, mm	0.50 – 0.75			✓	✓
Uniformity coefficient	< 1.60			✓	✓
Attrition sphericity, %	> 90			✓	✓

Note: 1) In plant inspection, each batch of product must be inspected.
2) In unscheduled sampling, one out of every 5 batches of product must be inspected.
3) An accompanying report must be provided for each batch of product.
4) The third-party inspection must be performed once for each batch.

6.35 氨水

6.35.1 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
NH3 含量/%	≥18	√		√	√
蒸发残渣/%	≤0.002			√	√
铅（Pb）	≤0.0005			√	√
还原高锰酸钾物质	≤0.0008			√	√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、供方每季度提供一次标准中要求全部检测项目的检测报告；
5、第三方检测项目，每年进行 1 次。

6.35 Aqueous ammonia

6.35.1 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
NH_3 content, %	≥ 18	✓		✓	✓
Evaporation residue, %	≤ 0.002			✓	✓
Lead (Pb)	≤ 0.0005			✓	✓
Potassium permanganate reducing substances	≤ 0.0008			✓	✓

Note: 1) In plant inspection, each batch of product must be inspected.
2) In unscheduled sampling, one out of every 5 batches of product must be inspected.
3) An accompanying report must be provided for each batch of product.
4) Suppliers must provide an inspection report for all inspection items required in the standards once per quarter.
5) The third-party inspection must be performed once per year for each batch.

000053

6.21　D001 大孔强酸性苯乙烯系阳离子交换树脂

6.21.1 参考 GB16579-1996-T 《D001 大孔强酸性苯乙烯系阳离子交换树脂》（见附件 21）。

6.21.2 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
外观	驼色至褐色球状不透明颗粒	√		√	√
含水量，%	45～55		√	√	√
质量全交换容量，mmol/g	≥4.00		√	√	√
体积全交换容量，mmol/ml	≥1.70		√	√	√
湿视密度，g/ml	0.75～0.85			√	√
湿真密度，g/ml	1.23～1.28			√	√
范围粒度%（粒径mm）	≥95（0.315～1.25）			√	√
下限粒度%（粒径mm）	≤1（≤0.315）			√	√
有效粒径，mm	0.40～0.70			√	√
均一系数	≤1.70			√	√
渗磨圆球率，%	≥85			√	√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、第三方检测项目，每批进行 1 次。

000054

6.21 D001 Macroporous acidic styrene series cation exchange resin

6.21.1 Refer to GB16579-1996-T "D001 Macroporous acidic styrene series cation exchange resin" (see Attachment 21).

6.21.2 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
Appearance	Brown to tawny opaque spherical particles	✓		✓	✓
Moisture, %	45 – 55		✓	✓	✓
Quality full exchange capacity, mmol/g	> 4.00		✓	✓	✓
Volume full exchange capacity, mmol/ml	> 1.70		✓	✓	✓
Wet superficial density, g/ml	0.75 – 0.85			✓	✓
Wet real density, g/ml	1.23 – 1.28			✓	✓
Granularity, % (grain size, mm)	> 95 (0.315 – 1.25)			✓	✓
Lower-limit granularity, % (grain size, mm)	< 1 (< 0.315)			✓	✓
Effective grain size, mm	0.40 – 0.70			✓	✓
Uniformity coefficient	< 1.70			✓	✓
Attrition sphericity, %	> 85			✓	✓

Note: 1) In plant inspection, each batch of product must be inspected.
2) In unscheduled sampling, one out of every 5 batches of product must be inspected.
3) An accompanying report must be provided for each batch of product.
4) The third-party inspection must be performed once for each batch.

000055

6.18 柠檬酸

6.18.1 参考 GB1987-2007 《食品添加剂 柠檬酸》（见附件 19）。

6.18.2 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
感官要求	无色或白色结晶颗粒或粉末，无臭，味极酸	√		√	√
含量，%	99.5～100.5			√	√
透光率，%	≥95.0	√		√	√
水分，%	7.5～9.0			√	√
易炭化物	≤1.0			√	√
硫酸灰分，%	≤0.05		√		√
氯化物，%	≤0.005				√
硫酸盐，%	≤0.015				√
草酸盐，%	≤0.01				√
钙盐，%	≤0.02				√
铁 mg/kg	≤5				√
砷盐 mg/kg	≤1				√
铅 mg/kg	≤0.5				√
水不溶物	过滤时间不超过 1min，滤膜基本不变色，目视肉眼可见杂质不超过 3 个		√	√	√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、供方每季度提供一次标准中要求全部检测项目的检测报告；
5、第三方检测项目，每年进行 1 次。

6.18 Citric acid

6.18.1 Refer to GB1987-2007 "Food additives – citric acid" (see Attachment 19).

6.18.2 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
Organoleptic requirements	Colorless or white crystal granules or powder, odorless, sour taste	✓		✓	✓
Content, %	99.5 – 100.5			✓	✓
Transmittance, %	> 95.0	✓		✓	✓
Moisture, %	7.5 – 9.0			✓	✓
Readily carbonizable substance	< 1.0			✓	✓
Sulfated ash, %	< 0.05		✓		✓
Chlorides, %	< 0.005				✓
Sulfates, %	< 0.015				✓
Oxalates, %	< 0.01				✓
Calcium salts, %	< 0.02				✓
Iron, mg/kg	< 5				✓
Arsenates, mg/kg	< 1				✓
Lead, mg/kg	< 0.5				✓
Water- insoluble matter	Filter time not to exceed 1 min, filter membrane essentially does not change color, no more than 3 impurities can be seen with the naked eye		✓	✓	✓

Note: 1) In plant inspection, each batch of product must be inspected.
2) In unscheduled sampling, one out of every 5 batches of product must be inspected.
3) An accompanying report must be provided for each batch of product.
4) Suppliers must provide an inspection report for all inspection items required in the standards once per quarter.
5) The third-party inspection must be performed once for each batch.

000057

6.34 消泡剂

6.34.1 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
感官	乳白色粘稠液体，无肉眼可见杂质，无异物，无异味	√		√	√
PH	6.0---8.0	√		√	√
不挥发物，%	≤30.0			√	√
稳定性（3000r/min,30min）	不分层				√
重金属(以 Pb 计) mg/kg	≤10.0				√
砷(以 As 计) mg/kg	≤0.5				√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、供方每季度提供一次标准中要求全部检测项目的检测报告；
5、第三方检测项目，每年进行 1 次。

6.34 Defoamer

6.34.1 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
Organoleptic	Milky white viscous liquid, no impurities visible to the naked eye, no foreign matter, no peculiar smell	✓		✓	✓
PH	6.0 – 8.0	✓		✓	✓
Non-volatile matter, %	< 30.0			✓	✓
Stability (3000 r/min, 30 min)	Not stratified				✓
Heavy metals (calculated as Pb), mg/kg	< 10.0				✓
Arsenic (calculated as Pb), mg/kg	< 0.5				✓

Note: 1) In plant inspection, each batch of product must be inspected.
2) In unscheduled sampling, one out of every 5 batches of product must be inspected.
3) An accompanying report must be provided for each batch of product.
4) Suppliers must provide an inspection report for all inspection items required in the standards once per quarter.
5) The third-party inspection must be performed once for each batch.

6.7 硫酸镁

6.7.1 参考 QB2555-2002《食用硫酸镁》（见附件 7）标准。

6.7.2 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
外观	白色细晶或粉末，无异味，无明显外来异物。	√		√	√
白度，度	≥75			√	√
硫酸镁，以 $MgSO_4 \cdot 7H_2O$ 计	≥99.30		√	√	√
水不溶物，%	≤0.05	√		√	√
氯化物（以 CL 计），%	≤0.1			√	√
澄清度试验	澄清	√		√	√
砷（As），mg/kg	≤0.5				√
铅（Pb），mg/kg	≤1.0				√
氟（F），mg/kg	≤15.0				√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、供方每季度提供一次标准中要求全部检测项目的检测报告；
5、第三方检测项目，每半年进行 1 次。

6.7 Magnesium sulfate

6.7.1 Refer to QB 2555-2002 "Magnesium sulfate for food" (see Attachment 7) standards.

6.7.2 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
Appearance	White crystals or powder, no peculiar odor, no obvious foreign matter	✓		✓	✓
Whiteness, degree	> 75			✓	✓
Magnesium sulfate, calculated as $MgSO_4 \cdot 7H_2O$	> 99.30		✓	✓	✓
Water-insoluble matter, %	< 0.05	✓		✓	✓
Chlorides (calculated as CL), %	< 0.1			✓	✓
Clarity test	Clear	✓		✓	✓
Arsenic (As), mg/kg	< 0.5				✓
Lead (Pb), mg/kg	< 1.0				✓
Fluorides (F), mg/kg	< 15.0				✓

Notes: 1) In plant inspection, each batch of product must be inspected.

2) In unscheduled sampling, one out of every 5 batches of product must be inspected.

3) An accompanying report must be provided for each batch of product.

4) Suppliers must provide an inspection report for all inspection items required in the standards once per quarter.

5) The-third party inspection must be performed once every six months for each batch.

6.33 磷酸二氢钾

6.33.1 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
外观	无色结晶	√		√	√
磷酸二氢钾含量，%	≥98.0			√	√
水不溶物，%	≤0.2		√	√	√
干燥失重，%	≤1.0	√		√	√
氟化物（以F计），mg/kg	≤10			√	√
砷（以As计），mg/kg	≤3				√
铅（Pb），mg/kg	≤5				√
重金属（以Pb计），mg/kg	≤20				√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、供方每季度提供一次标准中要求全部检测项目的检测报告；
5、第三方检测项目，每年进行 1 次。

6.33 Potassium dihydrogen phosphate

6.33.1 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third-Party Inspection
Appearance	Colorless crystals	✓		✓	✓
Potassium dihydrogen phosphate content, %	> 98.0			✓	✓
Water insoluble matter, %	< 0.2		✓	✓	✓
Weight loss on drying, %	< 1.0	✓		✓	✓
Fluorides (calculated as F), mg/kg	< 10			✓	✓
Arsenic (As), mg/kg	< 3				✓
Lead (Pb), mg/kg	< 5				✓
Heavy metals (calculated as Pb), mg/kg	< 20				✓

Notes: 1) In plant inspection, each batch of product must be inspected.
2) In unscheduled sampling, one out of every 5 batches of product must be inspected.
3) An accompanying report must be provided for each batch of product.
4) Suppliers must provide an inspection report for all inspection items required in the standards once per quarter.
5) The third-party inspection must be performed once for each batch.

6.32 酵母抽提物

6.32.1 参考 QB 23530- 2009 《酵母抽提物》（见附件 32）。

6.32.2 标准要求

质量项目	判定标准	到厂检测项目	不定期抽检项目	随货报告项目	第三方检测项目
色泽	黄棕或黄褐色	√		√	√
形态	膏状	√		√	√
气味	无异味	√		√	√
外观	无正常视力可见杂质	√		√	√
干物质（固形物）%	≥60.0	√		√	√
总氮（除盐干基计）%	≥9.0		√	√	√
氨基酸态氮（除盐干基计）%	≥3.0			√	√
pH	4.0-7.5	√		√	√
重金属（以Pb计）mg/kg	≤20				√
砷（以As计）mg/kg	≤2				√
菌落总数，cfu/ml	≤10000		√	√	√
大肠菌群，MPN/100g	≤30		√	√	√
沙门氏菌（25g样）	不得检出				√

注：1、到厂检测项目，每批到货必检；
2、不定期抽检项目，每 5 批到货至少进行 1 次；
3、随货报告项目，每批到货必须随货提供；
4、供方每季度提供一次标准中要求全部检测项目的检测报告；
5、第三方检测项目，每半年进行 1 次。

000064

6.32 Yeast extract

6.32.1 Refer to QB 23530-2009 "Yeast extract" (see Attachment 32)

6.32.2 Standards requirements

Quality Item	Determination Standards	Plant Inspection	Unscheduled Sampling	Accompanying Report	Third Party Inspection
Color and luster	Yellowish brown or tawny	✓		✓	✓
Form	Paste	✓		✓	✓
Odor	Odorless	✓		✓	✓
Appearance	No visible foreign matter under normal sight	✓		✓	✓
Dry matter (solid matter), %	> 60.0	✓		✓	✓
Total nitrogen (calculated in desalting dry base), %	> 9.0		✓	✓	✓
Amino nitrogen (calculated in desalting dry base), %	> 3.0			✓	✓
pH	4.0 – 7.5	✓		✓	✓
Heavy metals (calculated as Pb), mg/kg	< 20				✓
Arsenic (calculated as As), mg/kg	< 2				✓
Total colony count, cfu/ml	< 10000		✓	✓	✓
Coliform, MPN/100 g	< 30		✓	✓	✓
Salmonella (25 g sample)	Not tested				✓

Notes: 1) In plant inspection, each batch of product must be inspected.
2) In unscheduled sampling, one out of every 5 batches of product must be inspected.
3) An accompanying report must be provided for each batch of product.
4) Suppliers must provide an inspection report for all inspection items required in the standards once per quarter.
5) The third-party inspection must be performed once every six months for each batch.

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Appendix D. Documentation of Suitability of Production Materials for Use in Foods

涉及饮用水卫生安全的国产产品
卫生许可批件

产 品 名 称：争光牌离子交换树脂

产 品 类 别：滤料类

申报单位名称：杭州争光树脂有限公司

批 准 文 号：　浙卫水字［2007］50049　号

经审查，该产品符合《生活饮用水卫生监督管理办法》的规定，现予以批准。产品自批准之日起有效期四年。

浙江省卫生厅

二〇〇七年八月[illegible]日

Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

BEST ORIGINAL COPY

Oct. 23 2007 14:13 P.

FAX NO. :

FROM :

Domestic Products Involving Drinking Water Sanitation and Safety

Hygiene License Document

Product Name : Zhengguang brand ion-exchange resin

Product type: Filter Material

Unit of Application: Zhejiang Zhengguang Industrial Co.,Ltd

Approval Number : 浙卫水字 [2007] S0049 号

After examination, this product is compliance with requirement of document << Drinking Water Supervision and Management Procedure>>. The application is approved. The period of validity is 4 years from date of approval.

浙江省卫生厅

二00七年 八 月 十五 日

食品卫生许可证

单位名称：

法定代表人或业主：

地　　址：

许可范围：

发证机关：

年　月　日

Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

BEST ORIGINAL COPY

Food Hygiene Licence

Manufacturer Name : Fujian Nanping Yuanli Active Carbon Co.,Ltd

Legal Representative :

Address : Laizhou Development Zone, Nanping, Fujian

Approval Range : Active carbon used for food industry production

Issuing authority

Expiry Date:

MINISTRY OF HEALTH OF THE PEOPLE'S REPUBLIC OF CHINA

Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

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全国工业产品生产许可证

安琪酵母股份有限公司

经审查，下列产品符合食品生产许可证发证条件，特发此证。

产品名称: 其他食品（食品加工用酵母）

住　　所: 湖北宜昌城东大道168号（原中南路24号）
生产地址: 湖北宜昌城东大道168号（原中南路24号）
检验方式: 自行检验
证书编号: QS4205 2801 0005
有效期至: 2012年11月8日　　二00九 年 十 月 日

有效期届满6个月前，企业应当提出换证申请。

No. 00446297

Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

BEST ORIGINAL COPY

National Industry Products Production Licence

Angel Yeast Co., Ltd

After examination, the products listed below meet the issuing requirements of food production licence,. Hereby issued this certificate.

Product Name: Other Food (Yeast Products for Food Process)

Office Address: 24 Zhorgnan Road Yichang Hubei P. R.China
Production Address: 24 Zhorgnan Road Yichang Hubei P. R.China
Testing Type : Self-Testing
Licence No.: QS4205 2801 0005
Expiry Date : 2012年11月8日

二00九 年十 月 日

No. 00446342

中华人民共和国

卫生登记证书

企业名称：连云港润普食品配料有限公司

企业地址：连云港市灌南经济技术开发区（西区）

登记编号：3200D00222

经审查，你单位符合出口食品生产企业卫生要求，予以登记。

登记品种：氯化钾、柠檬酸钾、山梨酸钾、磷酸二氢钙、磷酸三钙、磷酸二氢钾、磷酸氢二钾、磷酸三钾、磷酸氢二钠、丙酸钙

发证日期：2007 年 09 月 28 日

证书有效期至：2010 年 09 月 27 日

江苏出入境检验检疫局

BEST ORIGINAL COPY

The People's Republic of China

Hygiene Registration Certificate

Manufacturer Name: Lianyungang Mupro Fi Plant

Address: Guannan Lianyungang Economic Development Zone

Registration No.: 3200D00222

After examination, the products listed below meet the requirements of food production enterprise. Hereby issued registration certificate.

Registration Products: potassium chloride, potassium citrate, potassium sorbate, calcium dihydrogen phosphate, tricalcium phosphate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, tripotassium phosphate, disodium hydrogen phosphate, calcium propionate.

Issued Date: 2007 年 09 月 28 日

Expiry Date: 2010 年 09 月 27 日

江苏出入境检验检疫局

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食品卫生许可证

贴量化分级等级标志

苏卫食证字(2008)第 320000C-050081 号

单位名称：连云港迪康食品添加剂厂

法定代表人或业主：范益华

地　　址：连云港市新浦临洪西路31号

许可范围：食品添加剂生产加工(详见副本)

发证机关(章)

二〇〇八年十一月二十日

有效期限：自2008年11月20日至2012年11月10日

Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

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食品卫生许可证（副本）

苏卫食证字（2008）第320000-050081号

单位名称：连云港迪康食品添加剂厂

法定代表人或业主：范益华

地　　址：连云港市新浦经济西路31号

许可范围：食品添加剂生产加工

产品品种名单：食品添加剂氯化钾、氯化镁、磷酸镁***

发证机关（章）

二〇〇八年十一月二十日

有效期限：自2008年11月20日至2012年11月19日

Prepared for Baolingbao Biology Co. Ltd
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Food Hygiene Licence

加贴
量化分级
等级标志

Licence No.: 苏卫食证字(2008)第 320000-065081 号

Manufacturer Name : Lianyungang Dikang Food Additive Plant

Legal Representative : 范益华

Address : Linhong West Road 31 Xinpu Lianyungang

Approval Range : Production and Process of Food Additive (Show in Duplicate)

发证机关(章)

二〇〇八 年十一 月 二十 日

Expiry Date: 自 2008年11月20日 至 2012年11月19日

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Food Hygiene Licence (Duplicate)

Licence No.: 苏卫食证字(2008)第320000-050081号

Manufacturer Name : Lianyungang Dikang Food Additive Plant

Legal Representative : 范益华

Address : Linhong West Road 31 Xinpu Lianyungang

Approval Range : Production and Process of Food Additive (Show in Duplicate)

Products list: Food additive potassium chloride, magnesium chloride, magnesium sulfate.

***

发证机关（章）

二〇〇八年十一月二十日

Expiry date : 自2008年11月20日至2012年11月19日

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Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

全国工业产品生产许可证

藁城市天坡化工有限公司

经审查，下列产品符合取得生产许可证发证条件，特发此证。

产品名称：无机化工碱性氨水

住　　所：藁城市尚书庄

生产地址：藁梅路工业园区

检验方式：自行检验

证书编号：QS1305 2302 0016

有效期至：2010年12月04日

二〇〇七年　　月　　日

Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

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No.K030456-2010

山东省产品质量监督检验研究院

Shandong Supervision and Inspection Institute for Product Quality

检 验 报 告

Test Report

共 2 页第 1 页

样品名称 Sample	[illegible]（分析纯）	检验类别 Test Kind	委托
送检单位 Client	藁城市天城化工有限公司	型号规格 Model, Type	/
生产单位 Manufacturer	藁城市天城化工有限公司	样品等级 Grade	/
送检单位地址 Address of Client	河北省石家庄市藁城市[illegible]	商 标 Brand	/
抽样地点 Sampling Place	/	送样人员 Client Sampler	送样
抽样基数 Sample Batch	/	接样日期 Receipt Date	2010-01-26
样品数量 Sample Quantity	1000mL	生产日期 Production Date	2010-01-22
样品特性和状态 Sample Description	液体、散装	样品批号 Batch No.	/
检验环境 Environmental Condition	[illegible]	检验日期 Test Date	2010.01.28--02.03
检验依据 Test Standard	按GB/T601-2007标准		
样品名称 Sample	[illegible]		
检验结论 Test Conclusion	该样品按GB/T601-2007标准检验合格		
备 注 Note	1. 本报告含封面及封二。		

批准：[illegible] 日期：2010-02-3

审核：[illegible] 日期：2010-02-3

主检：[illegible] 日期：2010-02-3

（检验单位章）

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No.C050458-2019

山东省产品质量监督检验研究院
检 验 报 告（续页）

共 2 页第 2 页

序号	[illegible]		标准	结果	结论
1	含量（NH3），	%	≥18	18.2	合格
2	蒸发残渣，	%	≤0.002	0.0001	合格
3	氯化物（CL），	%	≤0.00005	<0.00003	合格
4	硫化物（S），	%	≤0.0002	<0.00001	合格
5	硫酸盐（SO4）	%	≤0.001	<0.00001	合格
6	碳酸盐（以CO2计），	%	≤0.001	<0.00001	合格
7	磷酸盐（PO4），	%	≤0.00001	<0.00001	合格
8	钠（Na），	%	≤0.00005	<0.0002	合格
9	镁（Mg）	%	≤0.00001	<0.00001	合格
10	钾（K）	%	≤0.00001	<0.00001	合格
11	钙（Ca）	%	≤0.00001	<0.00001	合格
12	铁（Fe）	%	≤0.00002	<0.00001	合格
13	铜（Cu）	%	≤0.00001	<0.00001	合格
14	铅（Pb）		≤0.0005	<0.00001	合格
15	还原高锰酸钾物质		≤0.0008	<0.0001	合格

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000081

National Industry Products Production Licence

Gaocheng Tianpo Chemical Co.,Ltd.

After examination , the product listed below meets the licence-issued requirements. Hereby issued this certificate.

Product Name: aqueous ammonia

Office Address: Shangshu, Gaocheng

Production Address: Gaocheng Industrial Zone

Testing Type: Self-Testing

Licence No.: QS1305 2302 0016

Expiry Date: 2010年12月04日

二〇〇七年十二月五日

Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

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No. C050456/2010

山东省产品质量监督检验研究院

Shandong Supervision and Inspection Institute for Product Quality

检 验 报 告

Test Report

样品名称 Sample	[illegible](分析纯) equable ammonia(analytical reagent)	检验类别 Test Kind	委托
送检单位 Client	藁城市天坡化工有限公司 Gaocheng Tianpo Chemical Co.,Ltd	型号规格 Model, Type	/
生产单位 Manufacturer	藁城市天坡化工有限公司 Gaocheng Tianpo Chemical Co.,Ltd	样品等级 Grade	/
送检单位地址 Sender's Address	河北省石家庄市藁城市[illegible] Shangshu Gaocheng Shijiazhuang Hebei	商 标 Brand	/
抽样地点 Sampling Place	/	抽样人员 Sampler	王[illegible]
抽样基数 Sample Batch	/	抽样日期 Sampling Date	2010-01-26
样品数量 Sample Quantity	1000ml.	生产日期 Producing Date	2010-01-22
样品特性和状态 Sample Description	[illegible]、[illegible]	样品编号 Batch No.	/
检验环境 Environmental Condition	[illegible]	检验日期 Test Date	2010.01.28--02.03
检验依据 Test Standard	GB/T631-2007		
样品名称 Sample	[illegible] All items		
检验结论 Test Conclusion	该样品按GB/T631-2007标准[illegible]合格。 According to GB/T631-2007, the product is eligible.		
备 注 Note	1. 本报告有封面及封二。		

经理: Approved by 日期: 2010-02-3

审核: Audited by 日期: 2010-02-3

主检: Checked by 日期: 2010-02-3

(检验单位章)

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No.C050456-2019

山东省产品质量监督检验研究院

Shandong Supervision and Inspection Institute for Product Quality

检 验 报 告

Test Report

共 2 页第 2 页

序号	Item	标准 Standard	结果 Result	结论 Conclusion
1	含量（NH3）， %	≥18	18.2	合格
2	蒸发残渣 residue on evaporation %	≤0.002	0.0001	合格 eigible
3	氯化物（CL）， %	≤0.00005	<0.00003	合格 eigible
4	硫化物（S）， %	≤0.00002	<0.00001	合格 eigible
5	硫酸盐（SO4） %	≤0.001	<0.0001	合格 eigible
6	碳酸盐（以CO2计）， %	≤0.001	<0.0001	合格 eigible
7	磷酸盐（以P计）， %	≤0.00001	<0.00001	合格 eigible
8	钠（Na）， %	≤0.00005	<0.0002	合格 eigible
9	镁（Mg） %	≤0.00001	<0.00001	合格 eigible
10	钾（K）	≤0.00001	<0.00001	合格 eigible
11	钙（Ca）	≤0.00001	<0.00001	合格 eigible
12	铁（Fe） %	≤0.0002	<0.00001	合格 eigible
13	铜（Cu） %	≤0.0001	<0.00001	合格 eigible
14	铅（Pb）	≤0.0005	<0.00001	合格 eigible
15	还原高锰酸钾物质 Reduced potassium permanganate substances	≤0.0008	<0.0001	合格 eigible

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食品卫生许可证

（副本）

鲁卫食证字（ 2008 ）第370000-030021号

发证机关 山东省卫生厅

2008年8月12日

单位名称： 潍坊英轩实业有限公司

法定代表人或业主： 李世勇

地 址： 昌乐县城利民路西首

许可范围： 食品添加剂"柠檬酸、柠檬酸钠、柠檬酸钾"的生产、销售※

有效期限： 2006年 9 月 12日至 2010年 9 月 11日止

Food Hygiene Licence

(Duplicate)

Licence No.: 鲁卫食证字(2006)第370000-000021号

Issuing Authority: 山东省卫生厅

2006年9月12日

Manufacturer Name: Weifang Ensign Industry Co., Ltd,

Legal Representative: 李世勇

Address: The West End, Linin Road, Changle City, 262400, Shandong Province, China

Approval Range: Production and Sale of Food Additives Including Citric Acid, Sodium Citrate and

Expiry Date: 2006年9月12日至 2010年9月11日止

2009 Aug 05 15:18 HP LASERJET FAX P.6

Prepared for Baolingbao Biology Co. Ltd
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食品卫生许可证

（副 本）

鲁卫食证字（2009）第370000-000031号

发证机关 山东省卫生厅

2009年1月8日

（此证须每年审验方有效）

单位名称：烟台恒鑫化工科技有限公司

法定代表人或业主：张 玉

生产地址：烟台开发区滨江路12号华闻大厦

许可范围：复合食品添加剂"复合乳化硅油（聚二甲基硅氧烷、聚氧乙烯山梨醇酐单油酸酯）；复合消泡聚醚消泡剂（聚氧丙烯氧化乙烯甘油醚、聚氧乙烯聚氧丙烯季戊四醇醚）"的生产、销售

有效期限：2009年1月8日至2013年1月7日

验证日期：2009、2010、2011、2012年11月6日前

Prepared for Baolingbao Biology Co. Ltd
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Food Hygiene Licence

(Duplicate)

Licence No.: 鲁卫食证字（2009）第370000-000001号

Issuing Authority: [illegible]

([illegible])

Manufacturer's name: Yantai Tsinking Finechem Technology Co., Ltd.,

Legal Representative: [illegible]

Address: No.12 Ijiang road, Development Zone, Yantai

Approval Range: Production and Sale of Compound food additives "Compound Emulsified silicone oil" (Dimethicone, Polyoxyethylene sorbitan monooleate) and Compound Polyether Defoamer (Polyoxypropylene Oxyethylene Glycol, Polyoxyethylene Polyoxypropylene Pentaerythritol Ether).

Expiry Date: January 8, 2009 – January 7, 2013

Examination Date: Before November 6 of 2009, 2010, 2011 and 2012

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Prepared for Baolingbao Biology Co. Ltd
1002988.000 G0T0 0511 0001

(2005)量认(鲁)字(E0125)号　　CNAS L1461　　(2009)省质监认字18号

检 验 报 告

鲁冶质检（2010）★字 2010BH033 号

样品名称　不锈钢管

规格型号　DN150

委托单位　保龄宝生物股份有限公司

检验类别　委托检验

山东省黑色金属产品质量监督检验站

山 东 省 黑 色 金 属 产 品 质 量 监 督 检 验 站

检 验 报 告

№：2010BH033　　第 1 页 共 2 页

样品名称	不锈钢管		
规格型号	DN150	样品批号	/
委托单位	保龄宝生物股份有限公司		
生产(经销)单位	温州科宇		
样品来源	送样	样品数量	1 件
样品状态	管状	来样时间	2010.01.22.
检验类别	委托检验	检验时间	2010.01.29.
检验项目	化学成分		
检验地点	山东省黑色金属产品质量监督检验站		
检验依据	GB/T 20125-2006　GB/T 20122-2006　GB/T 223.11-2008		
检验结论	不锈钢管		
报告时间	2010 年 01 月 29 日		
备 注	/		

批准人：　　审核人：　　检验人：

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山东省黑色金属产品质量监督检验站检验报告附页

№: 2010BH032

样品名称（规格、型号）	不锈钢管 DN150				
序号	DN100 06Cr19Ni11	单位	标准要求	检验结果	单项判定
1	化学成分 C%	/	/	0.054	/
2	Si%	/	/	0.40	/
3	Mn%	/	/	1.03	/
4	P%	/	/	0.035	/
5	S%	/	/	0.003	/
6	Cr%	/	/	17.50	/
7	Ni%	/	/	8.21	/
8					
9	以下空白				
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(2005)量认(鲁)字(E0125)号

CNAS L1461

(2005)食质监认字18号

Testing Report

检 验 报 告

鲁冶质检（2010）字第 2010BH0531 号

Sample Name: stainless steel

Specification: DN150

consigner: Baolingbao Biology Co.,Ltd.

Classification of inspection: consigned inspection

Shandong Station of Ferrous Metal Product Quality Supervision and Inspection

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Shandong Station of Ferrous Metal Product Quality Supervision and Inspection

Testing Report

№: 2010BH033 第 1 页 共 2 页

Sample Name	stainless steel		
Specification	DN150	样品批号	/
consigner	Baolingbao Biology Co.,Ltd.		
Manufacturer	Wenzhou Keyu		
Source	Submitting sample for test	Quantity	1 件
Status	Pipe	Sampling Time	2010.01.22.
Classification of inspection	consigned inspection	Testing Time	2010.01.29.
Testing Items	chemical composition		
Testing Site	Shandong Station of Ferrous Metal Product Quality Supervision and Inspection		
Testing Standard	GB/T 20125-2006 GB/T 20123-2006 GB/T 223.11-2008		
Conclusion	不作结论。		
Report Time	2010 年 01 月 29 日		
Remarks	/		

Approved by: Audited by: Checked by:

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Shandong Station of Ferrous Metal Product Quality Supervision and Inspection

№ : 2010BH032

sample name	stainless steel DN150				
序号	DN100 06Cr19Ni11	Unit	Standard	Result	Conclusion
1	chemical composition C%	/	/	0.054	/
2	Si%	/	/	0.40	/
3	Mn%	/	/	1.03	/
4	P%	/	/	0.035	/
5	S%	/	/	0.003	/
6	Cr%	/	/	17.50	/
7	Ni%	/	/	8.21	/
8					
9	以下空白				
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Appendix E. Erythritol Batch Data

COA for 5 nonconsecutive production lots.

		Lot No.				
		110305/1	110313/3	110319/2	110326/1	110401/1
Test Item	**Standard**	**Results**				
Appearance	White crystalline powder	Eligible	Eligible	Eligible	Eligible	Eligible
Odor	Odorless	Eligible	Eligible	Eligible	Eligible	Eligible
Taste	Sweet and produces cooling sensation upon consumption	Eligible	Eligible	Eligible	Eligible	Eligible
pH	5.0 7.0	5.8	6.0	5.0	6.0	6.0
Melting point	119° -123°	120.8°	120.5°	120.9°	120.8°	120.7°
Assay %	99.5-100.5	100	100	100	100	100
Loss on drying %	<0.2	0.09	0.08	0.10	0.09	0.09
Lead ppm	<0.1	Eligible	Eligible	Eligible	Eligible	Eligible
Reducing sugar %	<0.3	Eligible	Eligible	Eligible	Eligible	Eligible
Residue on ignition %	<0.1	0.0038	0.0038	0.0037	0.0037	0.0039
Rihitol and Glycerol %	<0.1	Eligible	Eligible	Eligible	Eligible	Eligible
Cont of bacteria, cfu/g	<500	200	200	100	200	100
Yeast & Molc cfu/g	<50	10	20	5	10	5
Coliforms MPN/g	<10	Eligible	Eligible	Eligible	Eligible	Eligible
E.coli MPN/g	<3	Eligible	Eligible	Eligible	Eligible	Eligible

Bolibo 保龄宝 BAOLINGBAO BIOLOGY CO., LTD.
NO. 1DONGWAIHUAN ROAD,HI-TECH DEVELOPMENT ZONE,YUCHENG,SHANDONG,CHINA
TEL: +86-534-8918695 FAX: +86-534-2126095

CERTIFICATE OF ANALYSIS

Commodity	Erythritol	Batch Number	11030571
Manufacturing date	Mar.05,2011	Quantity (kg)	1
Expiry date	Mar.04,2013	Test date	Mar.05,2011
Test According As	QB/T 2985-2008		

Test Result

Serial Number	Test Item	Standard	Result
1	Appearance	White crystalline powder	Eligible
2	Odor	Odorless	Eligible
3	Taste	Sweet and produces cooling sensation upon consumption	Eligible
4	pH	5.0-7.0	5.8
5	Melting point	119° -123°	120.8°
6	Assay %	99.5-100.5	100
7	Loss on drying, %	<0.2	0.09
8	Lead ppm	<0.1	Eligible
9	Reducing sugar %	<0.3	Eligible
10	Residue on ignition %	<0.1	0.0038
11	Ribitol and Glycerol %	<0.1	Eligible
12	Cont of bacteria, cfu/g	<500	200
13	Yeast & Mold cfu/g	<50	10
14	Coliforms MPN/g	< 10	Eligible
15	E.coli MPN/g	<3	Eligible
Checker	02	Assessor	01

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Bolibo 保龄宝
BAOLINGBAO BIOLOGY CO., LTD.
NO.1DONGMAHUAN ROAD,HI-TECH DEVELOPMENT ZONE,YUCHENG,SHANDONG,CHINA
TEL: +86-534-8918695 FAX: +86-534-2126095

CERTIFICATE OF ANALYSIS

Commodity	Erythritol	Batch Number	11031373
Manufacturing date	Mar.13,2011	Quantity (kg)	1
Expiry date	Mar.12.2013	Test date	Mar.13.2011
Test According As	QB/T 2985-2008		

Test Result

Serial Number	Test Item	Standard	Result
1	Appearance	White crystalline powder	Eligible
2	Odor	Odorless	Eligible
3	Taste	Sweet and produces cooling sensaton upon consumption	Eligible
4	pH	5.0-7.0	6.0
5	Melting point	119° -123°	120.5°
6	Assay %	99.5-100.5	100
7	Loss on drying, %	<0.2	0.05
8	Lead ppm	<0.1	Eligible
9	Reducing sugar %	<0.3	Eligible
10	Residue on ignition %	<0.1	0.0038
11	Ribitol and Glycerol %	<0.1	Eligible
12	Cont of bacteria, cfu/g	<500	200
13	Yeast & Mold cfu/g	<50	20
14	Coliforms MPN/g	<10	Eligible
15	E.coli MPN/g	<3	Eligible
Checker	01	Assessor	02

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Bolibo 保龄宝
BAOLINGBAO BIOLOGY CO., LTD.
NO.1DONGWAIHUAN ROAD,H-TECH DEVELOPMENT ZONE,YUCHENG,SHANDONG,CHINA
TEL.: +86-534-8918695 FAX +86-534-2126095

CERTIFICATE OF ANALYSIS

Commodity	Erythritol	Batch Number	11031972
Manufacturing date	Mar.19,2011	Quantity (kg)	1
Expiry date	Mar.18,2013	Test date	Mar.19,2011
Test According As	QB/T 2985-2008		

Test Result

Serial Number	Test item	Standard	Result
1	Appearance	White crystalline powder	Eligible
2	Odor	Odorless	Eligible
3	Taste	Sweet and produces cooling sensation upon consumption	Eligible
4	pH	5.0-7.0	5.9
5	Melting point	119° -123°	120.9°
6	Assay %	99.5-100.5	100
7	Loss on drying, %	<0.2	0.10
8	Lead ppm	<0.1	Eligible
9	Reducing sugar %	<0.3	Eligible
10	Residue on ignition %	<0.1	0.0037
11	Ribitol and Glycerol %	<0.1	Eligible
12	Cont of bacteria, cfu/g	<500	100
13	Yeast & Mold cfu/g	<50	5
14	Coliforms MPN/g	<10	Eligible
15	E.coli MPN/g	<3	Eligible
Checker	02	Assessor	01

000098

Bolibo 保龄宝 BAOLINGBAO BIOLOGY CO., LTD.
NO.1 DONGMAIHUAN ROAD,HI-TECH DEVELOPMENT ZONE,YUCHENG,SHANDONG,CHINA
TEL: +86-534-8918695 FAX: +86-534-2126095

CERTIFICATE OF ANALYSIS

Commodity	Erythritol	Batch Number	11032671
Manufacturing date	Mar.26,2011	Quantity (kg)	1
Expiry date	Mar.25,2013	Test date	Mar.26,2011
Test According As	QB/T 2985-2008		

Test Result:

Serial Number	Test Item	Standard	Result
1	Appearance	White crystalline powder	Eligible
2	Odor	Odorless	Eligible
3	Taste	Sweet and produces cooling sensation upon consumption	Eligible
4	pH	5.0-7.0	6.0
5	Melting point	119° -123°	120.8°
6	Assay %	99.5-100.5	100
7	Loss on drying, %	<0.2	0.09
8	Lead ppm	<0.1	Eligible
9	Reducing sugar %	<0.3	Eligible
10	Residue on ignition %	<0.1	0.0037
11	Ribitol and Glycerol %	<0.1	Eligible
12	Cont of bacteria, cfu/g	<500	200
13	Yeast & Mold cfu/g	<50	10
14	Coliforms MPN/g	<10	Eligible
15	E.coli MPN/g	<3	Eligible
Checker	02	Assessor	01

Bolibo
BAOLINGBAO BIOLOGY CO., LTD.
NO. [illegible] HUANROAD HI TECH DEVELOPMENT ZONE FUCHENSHIHENGDONG,CHINA
TEL. [illegible] FAX: [illegible]

CERTIFICATE OF ANALYSIS

Commodity	Erythritol	Batch Number	19040171
Manufacturing date	Apr.1,2011	Quantity (kg)	1
Expiry date	Mar.31,2013	Test date	Apr.1,2011
Test According As	GB/T 2985-2008		

Test Result

Serial Number	Test Item	Standard	Result
1	Appearance	White crystalline powder	Eligible
2	Odor	Odorless	Eligible
3	Taste	Sweet and produces cooling sensation upon consumption	Eligible
4	pH	5.0-7.0	6.0
5	Melting point	119°-123°	120.7°
6	Assay %	99.5-100.5	100
7	Loss on drying, %	<0.2	0.09
8	Lead ppm	<0.1	Eligible
9	Reducing sugar %	<0.3	Eligible
10	Residue on Ignition %	<0.1	0.0039
11	Ribitol and Glycerol %	<0.1	Eligible
12	Cont of bacteria, cfu/g	<500	100
13	Yeast & Mold cfu/g	<50	5
14	Coliforms MPN/g	<10	Eligible
15	E.coli MPN/g	<3	Eligible
Checker	02	Assessor	01

000100

Bolibo 保龄宝 BAOLINGBAO BIOLOGY CO., LTD.
NO.1DONGWAIHUAN ROAD,HI-TECH DEVELOPMENT ZONE,YUCHENG,SHANDONG,CHINA
TEL: +86-534-8918685 FAX: +86-534-2126095

Analytical Method for All Tests Conducted On Product

Product: Erythritol

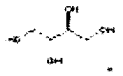
Test Item	Analytical Method
Appearance, Color, Odor ,Taste	Visual and Sensory Observation
Assay	High performance liquid chromatography method
Heavy metals	Atomic absorption spectometry
Loss on drying	Gravimetric method
PH	Acidometer
Reducing sugar	Fehling's test
Ash	Heating by Muffle furnace and weighing
Melting point	Meting point meter
Ribitol +glycerol	High performance liquid chromatography method
Cont of bacteria	Plate count method
Coliform	MPN method
Mold & Yeast	Plate count method
Partical size	Sieve analysis

Appendix F. Erythritol Monograph, FCC 6

Erythritol

Change to read:

1,2,3,4-Butanetetrol
meso-Erythritol



$C_4H_{10}O_4$ Formula wt 122.12

▲INS: 968▲FCC6 CAS: [149-32-6]

DESCRIPTION

Erythritol occurs as white crystals. It is obtained from the fermentation broth of the yeast *Moniliella pollinis* or *Trichosporonoides megachilensis*. It is stable to heat and is nonhygroscopic. It is soluble in water and is slightly soluble in alcohol. Erythritol melts between 119° and 123°.

Function Flavor enhancer; humectant; nutritive sweetener; texturizing agent; stabilizer

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

- **PROCEDURE**

  Acceptance criterion: The retention time of the major peak in the chromatogram of the *Sample solution* corresponds to that in the chromatogram of the *Standard solution* obtained in the *Assay* (below).

ASSAY

Change to read:

- **PROCEDURE**

  Mobile phase: Twice-distilled water

  Standard solution: ▲5.0 mg/mL erythritol standard[1] on the dried basis, 0.5 mg/mL glycerol, 0.5 mg/mL ribitol in *Mobile phase*▲FCC6

  [NOTE: Save this preparation for the test for *Ribitol and Glycerol* (below).]

  Sample solution: ▲Transfer 4.0 g of sample into a 25-mL volumetric flask, dissolve in and dilute to volume with *Mobile phase* and mix.▲FCC6 Pass through a 0.45-µm filter before injecting into the chromatograph.

  [NOTE: Save this preparation for the test for *Ribitol and Glycerol* (below).]

  Chromatographic system, Appendix IIA

  Mode: High-performance liquid chromatography with a constant-flow, pulseless pump

  Detector: Differential refractive index

  Column: Strong cation exchange resin in the hydrogen form consisting of a macroreticular sulfonated polystyrene divinylbenzene and an 8% crosslinked copolymer, such as MCI-CKOBSH or Shodex KC811 (Mitsubishi Chemical Corp. and Showa Denko, Ltd.), or equivalent.

  Flow rate: About 0.6 mL/min and maximum pressure system is about 1500 psi

  Injection volume: About 10 µL

  Analysis: Separately inject the *Standard solution* followed by the *Sample solution*, into the chromatograph and record the peak responses over a period of 60 min. The relative retention times are 1.0 for erythritol, 1.1 for glycerol, and 0.9 for ribitol. Calculate the percentage of erythritol in the sample using the following ▲formula.

$$2.5(C/W)\,(r_U / r_S)$$

  C = Concentration, in mg/mL, of erythritol in the *Standard solution*

  W = Weight, in g, of sample taken to prepare the *Sample solution*

  r_U = Peak response for erythritol from the *Sample solution*

  r_S = Peak response for erythritol from the *Standard solution*▲FCC6

  Acceptance criteria: NLT 99.5% and NMT 100.5% of $C_4H_{10}O_4$, calculated on the dried basis.

IMPURITIES

Inorganic Impurities

- **LEAD,** *Lead Limit Test, Atomic Absorption Spectrophotometric Graphite Furnace Method, Method I,* Appendix IIB

  Sample: 5 g

  Acceptance criterion NMT 1 mg/kg

Change to read:

Organic Impurities

- **RIBITOL AND GLYCEROL**

  Analysis: Determine as directed in the *Assay*. Identify the peak area responses for glycerol and ribitol in the chromatogram of the *Sample solution* by comparison with the chromatogram of the *Standard solution*, and calculate the percentage of glycerol and ribitol by the ▲formula:

$$2.5(C/W)(r_U / r_S)$$

  C = concentration, in mg/mL, of glycerol or ribitol in the *Standard solution*

  W = Weight, in g, of sample taken to prepare the *Sample solution*

  r_U = Peak response of glycerol or ribitol obtained from the *Sample solution*

  r_S = Peak response of glycerol or ribitol obtained from the *Standard solution*▲FCC6

  Acceptance criterion: The sum of glycerol and ribitol is NMT 0.1%.

SPECIFIC TESTS

- **LOSS ON DRYING,** Appendix IIC (105° for 4 h)

  Acceptance criterion: NMT 0.2%

- **REDUCING SUGARS (AS GLUCOSE)**

  Sample solution: Dissolve 500 mg of sample in 2 mL of water in a 20-mL flask, and mix.

  Control solution: Transfer 2 mL of a glucose solution containing 0.75 mg/mL into a 20-mL flask.

  Analysis: Add 1 mL each of *Fehling's Solution A* and of *Fehling's Solution B* (see cupric tartrate TS, alkaline,

[1] Available from Cerestar, EBS Vilvoorde R&D Centre, Centre of Expertise Fermentation, Havenstraat 84, 1800 Vilvoorde, Belgium; Mitsubishi Chemical Corporation, Performance Products Division, Food Ingredients Department, 11-1, Shiba-koen, 2-chrome, Minato-ku, Tokyo 105-0011, Japan

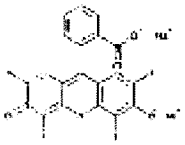
000102

under Solutions and Indicators) to both the *Sample solution* and the *Control solution*, heat to boiling, and cool.
Acceptance criterion: The resulting *Sample solution* is less turbid than the resulting *Control solution*, which forms a red-brown precipitate. (NMT 0.3%)

- **Residue on Ignition (Sulfated Ash)**, Appendix IIC
  Sample: 2 g
  Acceptance criterion: NMT 0.1%

Erythrosine[1]

CI Food Red 14
CI 45430
Class: Xanthene



$C_{20}H_6O_5I_4Na_2$ Formula wt 879.86

INS: 127 CAS: [16423-68-0]

DESCRIPTION

Erythrosine occurs as a brown powder or granules. It is principally the disodium salt of the monohydrate of 9-(*o*-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3*H*-xanthen-3-one. It dissolves in water to give a solution red at neutrality, with a yellow-brown precipitate in acid, and with a red precipitate in base. When dissolved in concentrated sulfuric acid, it yields a brown-yellow solution that evolves iodine and a precipitate of the free acid when heated. It is insoluble in ethanol.

Function Color

Packaging and Storage Store in well-closed containers.

IDENTIFICATION

- **Procedure**
  Sample solution: 2.8 µg/mL
  Analysis: Adjust the pH of two aliquots of the *Sample solution* to pH 7 and pH 13. Measure the absorbance intensities (*A*) and wavelength maxima of these solutions with a suitable UV-visible spectrophotometer.
  Acceptance criteria: In neutral and alkaline solutions, *A* = 0.32 at 527 nm with a shoulder at 490 nm. In acid solution, a yellow-brown precipitate forms.

ASSAY

- **Total Color**, *Colors, Methods I and III*, Appendix IIIC
  (Both methods must be used.)
  Method I (Spectrophotometric)
  Sample: 75 to 100 mg
  Analysis: Transfer the *Sample* into a 1-L volumetric flask; dissolve in and dilute to volume with water. Determine as directed at 527 nm using 0.110 L/(mg·cm) for the absorptivity (*a*) for Erythrosine.
  Method III (Gravimetric)
  Analysis: Determine as directed using 1.074 as the gravimetric conversion factor (*F*) for Erythrosine.
  Acceptance criterion: The average of results obtained from *Methods I and III* is NLT 87.0% total coloring matters.

IMPURITIES

Inorganic Impurities

- **Arsenic**, *Arsenic Limit Test*, Appendix IIIB
  Sample solution: Prepare as directed for organic compounds.
  Acceptance criterion: NMT 3 mg/kg
- **Lead**, *Lead Limit Test*, Appendix IIIB
  Sample solution: Prepare as directed for organic compounds.
  Control: 10 µg Pb (10 mL of *Diluted Standard Lead Solution*)
  Acceptance criterion: NMT 10 mg/kg

Organic Impurities

- **Uncombined Intermediates and Products of Side Reactions**, *Colors, Method I*, Appendix IIIC
  Sample solution: 20 mg/mL
  Analysis: Calculate the concentrations of 2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid, iodine, phthalic acid, sodium iodide, and triiodoresorcinol, using the following absorptivities.
  2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic acid: *a* = 0.047 L/(mg·cm) at 346 nm (alkaline solution)
  Iodine: *a* = 0.082 L/(mg·cm) at 245 nm (acidic solution)
  Phthalic acid: *a* = 0.045 L/(mg·cm) at 228 nm (acidic solution)
  Sodium iodide: *a* = 0.091 L/(mg·cm) at 220 nm (acidic solution).
  Triiodoresorcinol: *a* = 0.079 L/(mg·cm) at 223 nm (acidic solution)
  Acceptance criteria
  2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic acid: NMT 0.2%
  Sodium iodide: NMT 0.4%
  Triiodoresorcinol: NMT 0.2%
  Unhalogenated intermediates: Total NMT 0.1%

[1] To be used or sold for use in food that is marketed in the United States, this color additive must be from a batch that has been certified by the U.S. Food and Drug Administration (FDA). If it is not from an FDA-certified batch, it is not a permitted color additive for food use in the United States, even if it is compositionally equivalent. The name FD&C Red No. 3 can be applied only to FDA-certified batches of this color additive. Erythrosine is a common name given to the uncertified colorant. See the monograph entitled *FD&C Red No. 3* for directions for producing an FDA-certified batch.

Appendix G. Comparison with Erythritol Standard

高效液相色谱检测保龄宝赤藓糖醇

流动相： 二次蒸馏水

标准品溶液制备： 称量赤藓糖醇标准品[1] 0.2623g 全部移入 50ml 容量瓶；再分别称量核糖醇标准品 0.0245g 和丙三醇标准品[2] 0.0339g 移入同一容量瓶，用二次蒸馏水完全溶解，定容至刻度。

样品溶液制备： 称量赤藓糖醇样品[3] 3.9893g，全部移入 25ml 容量瓶，用二次蒸馏水完全溶解，定容至刻度，用 0.45 微米膜过滤备用。

备注： 以上使用的赤藓糖醇均经过 105 摄氏度 4 个小时的烘干

色谱系统： 高效液相色谱仪配有 Waters2410 示差折光检测器. 色谱柱：shodex RSpak KC-811（8mm×300mm）；流速：0.6mL/min；柱温：40 摄氏度；进样量：10 微升；压力：不高于 300psi。

测定： 色谱系统稳定后，分别注入上述制备的标准品溶液和样品溶液各 10 微升，记录 60min 的色谱图. 赤藓糖醇，丙三醇和核糖醇的相对保留时间分别为 1.0，1.1 和 0.9. 标准品色谱图与样品色谱图分别如图 1，图 2 所示.

结果计算：

- 赤藓糖醇：计算样品中赤藓糖醇的比例用下列公式：

$$\text{结果} = 2.5\ (C/W)\ (R_u/R_s)$$

C = 标准品溶液赤藓糖醇浓度 mg/ml,

W = 制备样品溶液的样品重量 g

R_u = 样品溶液赤藓糖醇峰响应值

R_s = 标准溶液赤藓糖醇峰响应值

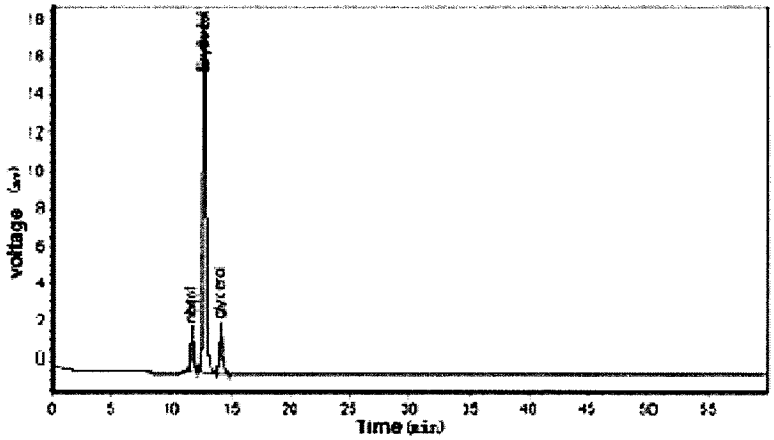
- 核糖醇和丙三醇： 如图 2 所示，核糖醇和丙三醇在该色谱条件下没有峰响应，其在样品中的含量低于该方法的检出限.

结论： 保龄宝产品与嘉吉标准样品等同，通过公式计算样品中赤藓糖醇含量为 99.94%，核糖醇+丙三醇的含量低于 0.1%。

---

1. 赤藓糖醇标准品来自 Cargill Polyols LLC，批次号：10G041BP952，相应 COA 见附件
2. 核糖醇和丙三醇标准品来自 Sigma Corporation
3. 赤藓糖醇样品来自保龄宝公司，批次号：10121773，相应 COA 见附件

000104

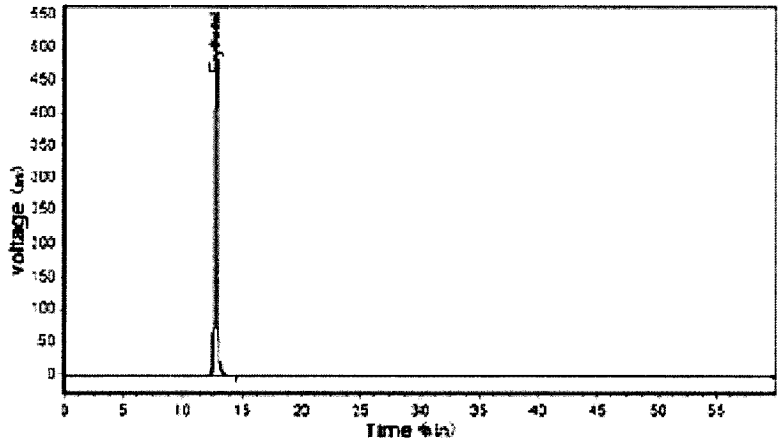


Analysis Result

Peak No.	Peak Name	Retention Time	Peak Height	Peak Area	Content
1	ribitol	11.840	1785.051	28379.520	7.8758
2	Erythritol	12.707	18397.213	297699.438	82.6695
3	glycerol	14.107	2019.816	34068.848	9.4547
Total			22212.679	360339.805	100.0000

图 1. 嘉吉赤藓糖醇添加丙三醇和核糖醇标准品

Fig.1 Chromatogram of Ribitol, Erythritol and Glycerol Standards.



Analysis Result

Peak No.	Peak Name	Retention Time	Peak Height	Peak Area	Content
1	Erythritol	12.707	537745.188	9056095.000	100.0000
Total			537745.188	9056095.000	100.0000

图 2.保龄宝赤藓糖醇

Fig. 2 Chromatogram of BLB Erythritol Sample

000105

Cargill Polyols LLC
650 Industrial Park Drive
PO Box 448
Blair, NE 68008
Fax: 1-402-535-1301
Tel: USA (877) 650-7080

Certificate of Analysis

Product	Zerose™ erythritol 16952
Lot Number	10G041BP952

PARAMETER	RESULT
Loss on Drying	0.06
Erythritol	99.95
Ribitol & Glycerol	0.06
pH	5.69

Date of Manufacture:	Aug 04 2010
Best Before Date:	Three Years from Date of Manufacture

000106

Bolibo 保龄宝
BAOLINGBAO BIOLOGY CO., LTD.
NO.1DONGWAIHUAN ROAD,HI-TECH DEVELOPMENT ZONE,YUCHENG,SHANDONG,CHINA
TEL: +86-534-8918695 FAX: +86-534-2126095

CERTIFICATE OF ANALYSIS

Commodity	Erythritol	Batch Number	10121773
Manufacturing date	Dec.17,2010	Quantity (kg)	Sample
Expiry date	Dec.16,2011	Test date	Dec.17,2010
Test According As	QB/T 2985-2008		

Test Result

Serial Number	Test Item	Standard	Result
1	Appearance	White crystalline powder	Eligible
2	Odor	Odorless	Eligible
3	Taste	Sweet and produces cooling sensation upon consumption	Eligible
4	pH	5.0-7.0	5.7
5	Melting point	119° -123°	120.6°
6	Assay %	99.5-100.5	99.94
7	Loss on drying, %	≤0.2	0.086
8	Lead ppm	≤1.0	Eligible
9	Reducing sugar %	≤0.3	Eligible
10	Residue on ignition %	≤0.1	0.0035
11	Ribitol and Glycerol %	≤0.1	Eligible
12	Cont of bacteria, cfu/g	≤500	150
13	Yeast & Mold cfu/g	≤50	10
14	Coliforms MPN/g	≤10	Eligible
15	E.coli MPN/g	≤3	Eligible
Checker	02	Assessor	01

NOTE: At the time of the analysis, the specification for lead was 1 ppm; the specification was subsequently revised to <0.1 ppm.

000107

High Performance Liquid Chromatography of Baolingbao Erythritol

Fluid phase: Double distilled water

Standard solution preparation: Weigh out 0.2623 g of erythritol standard product[1] and transfer the entire amount into a 50 ml flask, measure 0.0245 g of ribitol standard product and 0.0339 g of glycerol standard product[2] and transfer into the same flask. Dissolve thoroughly with double distilled water. Set a constant volume on the marked scale.

Sample solution preparation: Weigh out 3.9893 g of erythritol sample product[3] and transfer the entire amount into a 25 ml flask. Dissolve thoroughly with double distilled water and set a constant volume on the marked scale. Filter with a 0.45 μ membrane and set aside.

Notes: The erythritol used above has been dried over a stove at 105°C for 4 hr.

Chromatographic system: High performance chromatographer is joined with a Water 2410 differential refractive index detector. Chromatographic column: Shodex RSpak KC 811 (8 mm × 300 mm); flow rate: 0.6 ml/min; column temperature: 40°C; feed amount: 10 μl; pressure: no greater than 300 psi.

Determination: After the chromatographic system has stabilized, pour 10 μl each of the standard solutions and sample solution prepared above and record the chromatogram for 60 min. The corresponding retention times for erythritol, glycerol, and ribitol are 1.0, 1.1, and 0.9, respectively. The standard product chromatogram and the sample product chromatogram are shown in Figure 1 and Figure 2, respectively.

Calculation of results:

- Erythritol: Use the following formula to calculate the erythritol ratio in the sample product:

$$\text{Result} = 2.5\,(C/W)\,(R_u/R_s)$$

C = Standard solution erythritol concentration mg/ml,

W = Sample weight (g) of the prepared sample solution

R_u = Erythritol peak response value of the sample solution

1. Erythritol standard product is from Cargill Polyols LLC, batch number: 10G041BP952, see attachment for corresponding COA.
2. Ribitol and glycerol standard product are from Sigma Corporation.
3. Erythritol sample product is from Baolingbao Co., batch number: 10121773, see attachment for corresponding COA.

000108

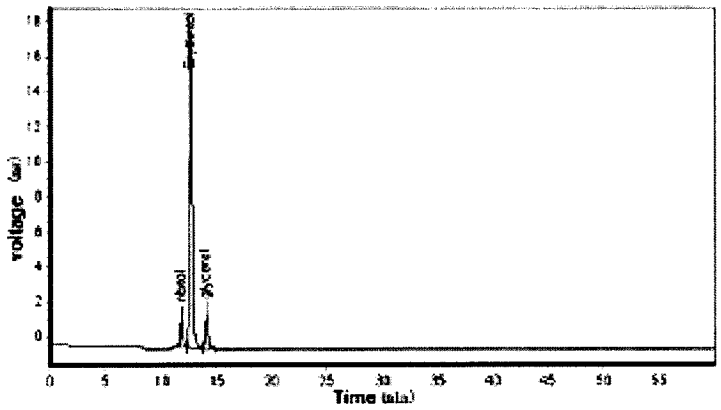
R_t = Erythritol peak response value of the standard solution

- Ribitol and glycerol: As shown in Figure 2, the ribitol and glycerol do not have a peak response under these chromatographic conditions. Their content in the sample product is less than can be measured by this method.

Conclusions: Baolingbao products and Cargill standard samples are the same. The erythritol content in the sample product as calculated by the formula is 99.94%, and the ribitol + glycerol content is less than 0.1%.

1. Erythritol standard product is from Cargill Polyols LLC. batch number: 10GC41BP952. see attachment for corresponding COA.
2. Ribitol and glycerol standard product are from Sigma Corporation.
3. Erythritol sample product is from Baolingbao Co., batch number: 10121773, see attachment for corresponding COA.

000109

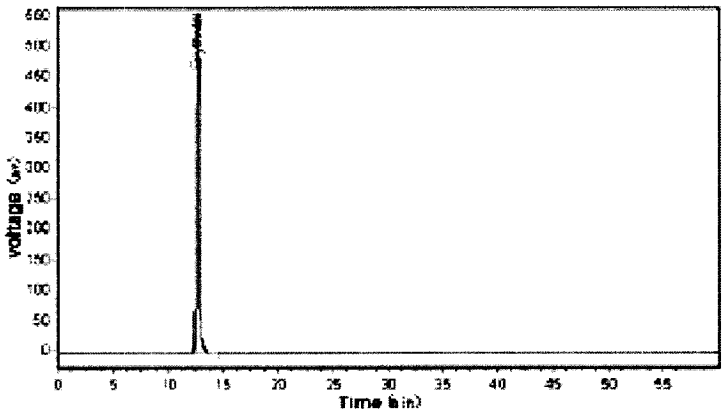


Analysis Result

Peak No.	Peak Name	Retention Time	Peak Height	Peak Area	Content
1	ribitol	11.840	1795.851	28379.520	7.8758
2	Erythritol	12.707	16597.213	297890.438	82.6696
3	glycerol	14.197	2919.816	34068.818	9.4547
Total			22312.679	360338.606	100.0000

Figure 1. Cargill Erythritol Added to Glycerol and Ribitol Standard Products

Figure 1 Chromatogram of Ribitol, Erythritol and Glycerol Standards



Analysis Result

Peak No.	Peak Name	Retention Time	Peak Height	Peak Area	Content
1	Erythritol	12.707	537745.188	9056098.000	100.0000
Total			537745.188	9056098.000	100.0000

Figure 2. Baolingbao Erythritol

Figure 2 Chromatogram of BLB Erythritol Sample

000110

Cargill Polyols LLC
650 Industrial Park Drive
PO Box 448
Blair, NE 68008
Fax (402) 533-1301
Tel: USA (877) 456-2000

Certificate of Analysis

Product	Zerose™ erythritol 16952
Lot Number	100041BP952

PARAMETER	RESULT
Loss on Drying	0.06
Erythritol	99.95
Ribitol & Glycerol	0.06
pH	5.69

Date of Manufacture:	Aug 04 2010
Best Before Date:	Three Years from Date of Manufacture

000111

Bolibo 保龄宝
BAOLINGBAO BIOLOGY CO., LTD.
NO.1DONGWAIHUAN ROAD HI-TECH DEVELOPMENT ZONE,YUCHENG,SHANDONG,CHINA
TEL: +86-534-8918695 FAX: +86-534-2125095

CERTIFICATE OF ANALYSIS

Commodity	Erythritol	Batch Number	10121773
Manufacturing date	Dec. 17, 2010	Quantity (kg)	Sample
Expiry date	Dec. 16, 2011	Test date	Dec. 17, 2010
Test according to	QB/T 2985-2008		

Test Result

Serial Number	Test Item	Standard	Result
1	Appearance	White crystalline powder	Eligible
2	Odor	Odorless	Eligible
3	Taste	Sweet and produces cooling sensation upon consumption	Eligible
4	pH	5.0-7.0	5.7
5	Melting point	119-123°	120.5°
6	Assay, %	99.5-100.5	99.94
7	Loss on drying, %	≤ 0.2	0.086
8	Lead, ppm	≤ 1.0	Eligible
9	Reducing sugar, %	≤ 0.3	Eligible
10	Residue on ignition, %	≤ 0.1	0.0035
11	Ribitol and glycerol, %	≤ 0.1	Eligible
12	Cont of bacteria, cfu/g	≤ 500	150
13	Yeast and mold, cfu/g	≤ 50	10
14	Coliforms, MPN/g	≤ 10	Eligible
15	E. coli, MPN/g	≤ 3	Eligible
Checker	02	Assessor	01

Appendix H. Identification of *Yarrowia lipolytica*

INSTITUTE OF MICROBIOLOGY
CHINESE ACADEMY OF SCIENCES

检 测 鉴 定 报 告
Identification Report Form

(2010)微检字第 0179 号 (No. 2010-)

送检单位: From:	保龄宝生物股份有限公司 Baolingbao Biology Co., Ltd.		
样品名称: Name of sample:	酵母菌培养物 Yeast culture		
样品数量: Amount & No.:	1 株 (1 strain) CGMCC No. 1431	送检时间: Date of acceptance:	2010/04/01
检测鉴定负责人(签字) Identified by (sign)	(b) (6)	检测鉴定操作人(签字) Tests performed by (sign)	(b) (6)

检测鉴定内容(Experiments performed):

对保龄宝生物股份有限公司送检的一株酵母菌 CGMCC No. 1431 进行了菌落和细胞形态学观察和核糖体 ITS 区序列测定，分析结果表明，送检菌株的形态特征与解脂假丝酵母(*Candida lipolytica*)的标准描述完全一致，其 ITS 区碱基序列与 *Candida lipolytica* ATCC 9773 的 ITS 碱基序列相似率为 100%(比较结果见附件 1)。据此，将送检酵母菌株做出了种的鉴定。

Morphological characterization: The culture is pure; colonies are cream, butyrous to membranous; vegetative cells are ellipsoidal to elongate and multilateral budding; pseudohyphae and true hyphae are present.

rDNA ITS sequence comparion: The ITS (ITS1-5.8S-ITS2) region sequence of strain CGMCC No. 1431 determined is identical to that of strain *Candida lipolytica* ATCC 9773 (see attachment 1)

检测鉴定结果(Result of species identification):

解脂假丝酵母 *Candida lipolytica* (F. C. Harrison) Diddens & Lodder

中国科学院微生物研究所
Institute of Microbiology, CAS (seal)

日期 Data: 2010/04/23

附件(attachment) 1.

送检菌株 CGMCC No. 1431 的 rDNA ITS 区碱基序列
ITS sequence of strain CGMCC No. 1431

```
gtaggtgaac ctgcggaagg atcattatig attttatcta tttctgtgga tttctattct
attacagcgt catcttatct caattctaac tatcaacaac ggatctcttg gctctcacat
cgatgaagaa cgcagcgaac cgcgatattt tttgtgactt gcagatgtga atcatcaatc
tttgaacgca catcgcgcgg tatggcattc cgtacccgcac ggatggaggg gcgtgttccc
tctgggatcg catcgcttcc ttgaaatgga ttttttaaac ctctcaattta ttagtcatt
tcacctccttcatccgagatt tacccgctga acttaagcat atcaataage
```

送检菌株与解脂假丝酵母 ATCC 9773 的 ITS 序列比较
rDNA ITS sequence comparison between strains CGMCC No. 1431 and ATCC 9773 (GenBank accession number: EU252546):

```
Query = CGMCC No. 1431, Sbjct = ATCC 9773; Score = 647 bits (350), Expect = 0.0
Identities = 350/350 (100%), Gaps = 0/350 (0%)

Query  1    GTAGGTGAACCTGCGGAAGGATCATTATTGATTTTATCTATTTCTGTGGATTTCTATTCT  60

Sbjct  1    GTAGGTGAACCTGCGGAAGGATCATTATTGATTTTATCTATTTCTGTGGATTTCTATTCT  60

Query  61   ATTACAGCGTCATTTTATCTCAATTATAACTATCAACAACGGATCTCTTGGCTCTCACAT  120

Sbjct  61   ATTACAGCGTCATTTTATCTCAATTATAACTATCAACAACGGATCTCTTGGCTCTCACAT  120

Query  121  CGATGAAGAACGCAGCGAACCGCGATATTTTTGTGACTTGCAGATGTGAATCATCAATC  180

Sbjct  121  CGATGAAGAACGCAGCGAACCGCGATATTTTTGTGACTTGCAGATGTGAATCATCAATC  180

Query  181  TTTGAACGCACATTGCGCGGTATGGCATTCCGTACCGCACGGATGGAGGGCGTGTTCCC  240

Sbjct  181  TTTGAACGCACATTGCGCGGTATGGCATTCCGTACCGCACGGATGGAGGGCGTGTTCCC  240

Query  241  TCTGGATCGCATTGCTTCTTGAAATGGATTTTTTTAAACTCTCAATTATTACGTCATT  300

Sbjct  241  TCTGGATCGCATTGCTTCTTGAAATGGATTTTTTTAAACTCTCAATTATTACGTCATT  300

Query  301  TCACCTCCTTCATCCGAGATTACCCGCTGAACTTAAGCATATCAATAAGC  350

Sbjct  301  TCACCTCCTTCATCCGAGATTACCCGCTGAACTTAAGCATATCAATAAGC  350
```

INSTITUTE OF MICROBIOLOGY
CHINESE ACADEMY OF SCIENCES

Identification Report Form

(2010) Weijianzi No. 073 (No. 2010-)
Page 1 of 1

Sent by: Baolingbao Biology Co., Ltd.
Sample name: Yeast culture
Sample quantity: 1 strain
CGMCC No. 1431 Date sent: 04/01/2010
Identified by (sign): [signature: illegible] Identification test performed by (sign): [signature: illegible]

Result of species identification:

Colony and cell morphological observations and ribosome ITS area sequencing were performed on a strain of yeast sent by Baolingbao Biology Co., Ltd. The analysis results showed that the morphological characteristics of the strain are entirely consistent with the *Candida lipolytica* standard description, and the base sequence rate of similarity of the ITS area with *Candida lipolytica* ATCC 9773 is 100% (see Attachment 1 for comparison results). Based on this, type identification was carried out on the yeast strain sent.

Morphological characterization: The culture is pure; colonies are cream, butyrous to membranous; vegetative cells are ellipsoidal to elongate and multilateral budding; pseudohyphae and true hyphae are present.

rDNA ITS sequence comparion: The ITS (ITS1-5.8S-ITS2) region sequence of strain CGMCC No. 1431 determined is identical to that of strain *Candida lipolytica* ATCC 9773 (see attachment 1)

[Seal: Chinese Academy of Sciences, Institute of Microbiology, Identification Testing Seal]

Identification Testing Results:

Candida lipolytica (F. C. Harrison) Diddens & Lodder

Chinese Academy of Sciences, Institute of Microbiology, CAS (seal)
[Seal: Chinese Academy of Sciences, Institute of Microbiology, Identification Testing Seal]

Date: 04/23/2010

000115

Attachment 1

rDNA ITS area sequence of strain CGMCC No. 1431

```
gtaggtgaac ctgcggaagg atcattattg attttatcta tttctgtgga tttctattct
attacagcgt cattttatct caattataac tatcaacaac ggatctcttg gctctcacat
cgatgaagaa cgcagcgaac cgcgatattt tttgtgactt gcagatgtga atcatcaatc
tttgaacgca cattgcgcgg tatggcattc cgtacccgcac ggatggagga gcgtgttccc
tctgggatcg cattgctttc ttgaaatgga tttttttaaa ctctcaattta ttacgtcatt
teacctcctt catccgagat taccccgctga acttaagcat atcaataagc
```

Sequencing comparison between sent strain and ATCC 9773 (GenBank accession number: EU252546):

```
Query  CGMCC No. 1431, Sbjct = ATCC 9773; Score = 647 bits (350), Expect = 0.0
Identities = 350/350 (100%), Gaps = 0/350 (0%)

Query  1    GTAGGTGAACCTGCTGAAGGATCATTATTGATTTTATCTATTTCTGTGGATTTCTATTCT  60
Sbjct  1    GTAGGTGAACCTGCTGAAGGATCATTATTGATTTTATCTATTTCTGTGGATTTCTATTCT  60

Query  61   ATTACAGCGTCATTTTATCTCAATTATAACTATCAACAACGGATCTCTTGGCTCTCACAT  120
Sbjct  61   ATTACAGCGTCATTTTATCTCAATTATAACTATCAACAACGGATCTCTTGGCTCTCACAT  120

Query  121  CGATGAAGAACGCAGCGAACGCGCGATATTTTTTGTGACTTGCAGATGTGAATCATCAATC  180
Sbjct  121  CGATGAAGAACGCAGCGAACGCGCGATATTTTTTGTGACTTGCAGATGTGAATCATCAATC  180

Query  181  TTTGAACGCACATTGCGCGGTATGGCATTCCGTACCGCACGGATGGAGGAGCGTGTTCCC  240
Sbjct  181  TTTGAACGCACATTGCGCGGTATGGCATTCCGTACCGCACGGATGGAGGAGCGTGTTCCC  240

Query  241  TCTGGGATCGCATTGCTTTCTTGAAATGGATTTTTTTAAACTCTCAATTATTACGTCATT  300
Sbjct  241  TCTGGGATCGCATTGCTTTCTTGAAATGGATTTTTTTAAACTCTCAATTATTACGTCATT  300

Query  301  TCACCTCCTTCATCCGAGATTACCCGCTGAACTTAAGCATATCAATAAGC  350
Sbjct  301  TCACCTCCTTCATCCGAGATTACCCGCTGAACTTAAGCATATCAATAAGC  350
```

[Seal: Chinese Academy of Sciences, Institute of Microbiology, Identification Testing Seal]

Fungi , Yeasts and Yeast Genetic Stock

ATCC® Number:	9773™	Order this item	Price:	$40.00

Preceptrol® Culture

Organism:	*Yarrowia lipolytica* (Wickerham et al.) van der Walt et von Arx, teleomorph deposited as *Mycoderma lipolytica*
Alternate State:	*Candida lipolytica* (Harrison) Diddens et Lodder, anamorph
Designations:	251 [CBS 2072, IFO 1742, KCM-0232]
Depositors:	PR Burkholder
Biosafety Level:	1
Shipped:	freeze-dried
Growth Conditions:	ATCC medium 200: YM agar or YM broth **Temperature:** 24.0°C
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	control strain for identification [36320] quality control strain [92180]
Comments:	Growth factor [490]
Subcollection:	Yeasts
References:	490: et al., Burkholder PR. Studies on some growth factors of yeasts. J. Bacteriol. 48: 385-391, 1944. 36320: Espinel-Ingroff A, et al. Comparison of RapID yeast plus system with API 20C system for identification of common, new, and emerging yeast pathogens. J. Clin. Microbiol. 36: 883-886, 1998. PubMed: 9542903 92180: VITEK YBC. :bioMerieux 16173321: Knutsen AK, et al. Polyphasic re-examination of Yarrowia lipolytica strains and the description of three novel Candida species: Candida oslonensis sp. nov., Candida alimentaria sp. nov. and Candida hollandica sp. nov. Int. J. Syst. Evol. Microbiol. 57: 2426-2435, 2007. PubMed: 17911320

Related Links ▸

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Products

000117

Appendix I. Safety Evaluation of Erythritol produced by *Yarrowia lipolytica* CGMCC No. 1431 Using the Pariza and Johnson (2001) Decision Tree

The decision tree published by Pariza and Johnson was developed to evaluate the safety of microbial enzyme preparations. In this instance it is used to evaluate the safety of erythritol that was produced by *Yarrowia lipolytica* CGMCC No. 143. Steps that do not directly pertain to this evaluation are omitted.

Step 1. Is the production strain genetically modified?
Answer: **NO** (if no, go to 6)

Step 6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?
Answer: **NO** (if no, go to 7)

Step 7. Is the organism nonpathogenic?
Answer: **YES** (if yes, go to 8)

Step 8. Is the test article free of antibiotics?
Answer: **YES** (if yes, go to 9)

Step 9. Is the test article free of oral toxins known to be produced by other members of the same species?
Answer: **YES** (if yes, go to 11)

Step 11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety?
Answer: **YES*** (if yes, the test article is ACCEPTED)

*The erythritol product is substantially equivalent to erythritol products already determined to be GRAS (filed as GRN 76 and GRN 208). Therefore, the erythritol "test article" meets the NOAEL requirement and is ACCEPTED.

Exhibits

Exhibit 1. Report of the Expert Panel

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE STATUS (GRAS) OF ERYTHRITOL FROM *YARROWIA LIPOLYTICA* IN SELECT FOODS

Introduction

The undersigned, an independent panel of experts, qualified by their scientific training and relevant experience to evaluate the safety of food and food ingredients, was requested by Baolingbao Biology Co., Ltd., to determine the safety and Generally Recognized as Safe (GRAS) status of erythritol, produced via the fermentation of glucose by *Yarrowia lipolytica*,[1] in select foods. The intended uses of erythritol produced from *Yarrowia lipolytica* are identical to the existing GRAS uses of erythritol produced from *Moniliella pollinis* or *Trichosporonoides megachiliensis* and are up to the specified maximum levels in the following categories of foods: reduced- and low calorie carbonated and non-carbonated beverages (3.5%); dairy drinks (chocolate and flavored milks) (3.5%); frozen dairy desserts (regular ice cream, soft serve, sorbet) (10%); puddings (instant, phosphate set) (10%); yogurt (regular and frozen) (10%); bakery fillings (fruit, custard, cream, pudding) (15%); cakes and cookies (regular and dietetic) (15%); fat-based cream used in modified fat/calorie cookies, cakes and pastries (60%); chewing gum (60%); soft candies (non-chocolate, plain chocolate, chocolate coated) (60%); hard candies (including pressed candy, mints and cough drops) (99%); and sugar substitutes (carrier) (100%). The functions of erythritol in food include flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant, and texturizer. Literature searches bearing on the safety of *Y. lipolytica* for use in the production of erythritol (through March 2011) were conducted by Exponent and these were summarized in a dossier "GRAS Determination for the Use of Erythritol from *Yarrowia lipolytica* in Select Foods". The safety information on erythritol reviewed in the previous determinations of GRAS status (filed as GRN 76 and GRN 208) was briefly summarized in the dossier. A search of PubMed for information related to the safety of erythritol published since 2006, the year of the most recent Notification to FDA of the GRAS status of erythritol under its intended conditions of use, also was conducted by Exponent and findings are summarized in the dossier. The Expert Panel members reviewed the safety

[1] The yeast *Yarrowia lipolytica* was previous classified as *Candida lipolytica*.

assessment dossier prepared by Exponent and other information deemed appropriate and necessary and agreed to the decision described herein.

Description

Erythritol ($C_4H_{10}O_4$; CAS No. 149-32-6), or 1,2,3,4-Butanetetrol, is a naturally occurring 4-carbon sugar alcohol that has a sweetness of approximately 60 to 80% that of sucrose. It is found in commonly consumed fermented foods and some fruits such as watermelon, pears, and grapes. Erythritol has a history of use as a food ingredient in Japan and other countries around the world. It is used in foods primarily as a low calorie sweetener, though it also provides technical functions in foods including flavor enhancer, formulation aid, humectant, stabilizer and thickener, sequestrant, and texturizer.

The safety of erythritol for use as an ingredient in food has been extensively reviewed and evaluated. The GRAS status of the uses of erythritol in a variety of foods, including uses identical to the uses proposed for erythritol produced from *Y. lipolytica*, was previously established based on information available in the publicly available literature. These levels of erythritol in the U.S. food supply were determined to be safe and first recognized as GRAS by a Panel of Experts qualified by scientific training and experience in 2001, and again in 2006. The erythritol product that is the subject of this GRAS determination meets appropriate food-grade specifications, currently published in the in the Food Chemical Codex ("FCC") VI (FCC, 2008) monograph and is substantially equivalent to the erythritol that was the subject of GRN 76 and GRN 208, both acceptable to the USFDA.

The yeast *Yarrowia lipolytica* was originally classified as *Candida* since no sexual state had been described. Following the identification of the perfect form (sexual stage), it was reclassified several times, and finally as *Yarrowia lipolytica*. The organism is referred to as both *Candida lipolytica* and *Yarrowia lipolytica* in the literature. Analysis of the genome sequence of *Y. lipolytica* revealed that it is very distantly related to other yeasts, and instead shares a number of common properties with filamentous fungi.

Yarrowia lipolytica has a history of consumption as a naturally occurring organism in various foods. *Y. lipolytica* is isolated from foods and natural environments. Food products in which the

organism has been found typically include products with a high concentration of fat and protein such as dairy products (including cheese), shoyu (soy sauce), poultry products, sausage, and salads containing meat or shrimp. *Yarrowia lipolytica* is permitted as a secondary direct food additive for fermentation production of citric acid that may be used in food for human consumption (21 CFR 173.165). The use of eicosapentaenoic acid (EPA)-containing oil derived from modified *Y. lipolytica* in select foods was recently determined to be GRAS by Du Pont Applied BioSciences (filed as GRN 355); FDA was notified of the determination and responded with a letter of no concern. The organism is used in the production of food flavors as well as numerous industrial applications.

Manufacturing Process

Erythritol is produced through fermentation of glucose catalyzed by the yeast *Y. lipolytica*. Following the fermentation, the resulting erythritol is purified through a series of filtration, concentration, and crystallization procedures. All materials used are documented as approved for food use.

The fermentation step begins with sterilization of the fermentation tank by steam. Glucose, yeast extract, KH_2PO_4, a defoamer, magnesium sulfate, and ammonium citrate (aqueous ammonia + citrate) are transferred to the fermentation tank via continuous sterilization equipment and *C. lipolytica* is transferred into the tank under aseptic conditions. Conversion of glucose to erythritol by *Y. lipolytica* occurs through an aerobic fermentation process. Throughout the fermentation, the temperature in the tank is maintained between 25 and 37°C.

Following the fermentation step, the fermentation solution is filtered through a membrane to completely remove microbial cells. The microorganisms captured in the membrane filtration are killed in a heat treatment step (80° C for 30 minutes). The resulting clear solution from the filtration step is concentrated under reduced pressure to a concentration of 40-60%. Through cooling the solution, the erythritol is crystallized. The erythritol crystals are then separated from the crystal-containing slurry via centrifugation. Activated carbon is added to the redissolved solution for decolorization, and the solution is demineralized using ion-exchange resins. The resulting liquid is then concentrated via a second concentration step followed by vacuum concentration and recrystallization. Erythritol crystals are separated from the crystal-containing

slurry via centrifugation and solid erythritol is obtained. A vibrating fluidized bed dryer is then used to dry the crystals, and a mesh screen is used to obtain crystals of the desired particle size. A magnetic separator is employed to ensure that no metal fragments are present in the erythritol crystals. The final erythritol product is packaged in bags. The erythritol product meets appropriate food-grade specifications, currently published in the in the Food Chemical Codex ("FCC") VI (FCC, 2008) monograph.

Functionality

The functions of erythritol in food included flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant, and texturizer. The intended uses of erythritol produced from *Yarrowia lipolytica* are identical to the existing GRAS uses of erythritol produced from *M. pollinis* or *T. megachiliensis* and are up to the specified maximum levels in the following categories of foods: reduced- and low calorie carbonated and non-carbonated beverages (3.5%); dairy drinks (chocolate and flavored milks) (3.5%); frozen dairy desserts (regular ice cream, soft serve, sorbet) (10%); puddings (instant, phosphate set) (10%); yogurt (regular and frozen) (10%); bakery fillings (fruit, custard, cream, pudding) (15%); cakes and cookies (regular and dietetic) (15%); fat-based cream used in modified fat/calorie cookies, cakes and pastries (60%); chewing gum (60%); soft candies (non-chocolate, plain chocolate, chocolate coated) (60%); hard candies (including pressed candy, mints and cough drops) (99%); and sugar substitutes (carrier) (100%).

Exposure

Given that the intended uses of erythritol are identical across all sources of erythritol (i.e., produced by fermenting glucose using either *C. lipolytica*, *M. pollinis*, or *T. megachiliensis)*, the previously calculated estimates of erythritol intake (2001 and 2006) provide valid estimates of potential intake of erythritol produced from *C. lipolytica*. The estimated average *per user* mean intake of all intended uses of erythritol is 13 g/day, and the *per user* 90th percentile intake is 30 g/day.[2]

[2] As calculated by FDA and noted in the response letter to GRN 76.

The maximum proposed use of erythritol in beverages including reduced- and low-calorie carbonated or noncarbonated beverages and dairy drinks [chocolate and flavored milks] is 3.5%. Assuming young children age 2-6 years consume a 240 mL beverage serving during an eating occasion, the maximum erythritol intake is 8.4 g per occasion, which is equivalent to 0.56 mg/kg bw per occasion assuming a default body weight of 15 kg. Likewise, assuming a person in the U.S. population age 2 years and older consumes two beverage servings during a single eating occasion (480 mL), the maximum erythritol intake is 16.8 g per eating occasion, or 0.28 mg/kg bw per eating occasion assuming a default body weight of 60 kg.

Safety Data

The safety of erythritol has been reviewed by the FDA, the WHO/FAO Joint Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF) of the European Union, and has been the subject of review by several GRAS Expert Panels. A comprehensive, critical review and summary of the biochemical, metabolic, toxicological, and clinical data was published by Munro et al (1998). The previous safety determinations of erythritol were based on published results of acute, subacute, and chronic oral toxicity studies in rats, mice, and dogs and studies conducted to assess the potential carcinogenicity, reproductive and developmental effects, genotoxicity, metabolic fate, and human tolerance.

Results of studies in both animals and humans demonstrated that erythritol is quickly absorbed, is not metabolized systemically, and is excreted unchanged in the urine. Based on studies in humans, approximately 90% of the ingested erythritol is absorbed, leaving up to 10% of the ingested erythritol potentially available for fermentation by the colonic microflora. Results from *in vitro* incubation studies suggest that colonic fermentation of erythritol in humans is unlikely. Because the absorbed portion of erythritol is not systemically metabolized, it does not contribute to the caloric load.

No adverse toxicological effects were reported in studies in which animals consumed up to 20% erythritol in the diet. Treatment of animals with high doses of erythritol was associated with some physiological changes including transient diarrhea, increased cecal and kidney weights, increased water consumption and urine output, and minor changes in some urinary parameters. No histopathological evidence of toxicity was observed, however, and the effects reversed after

cessation of exposure. The observed effects were considered to be physiological responses to high loads of poorly absorbed low molecular weight compounds and not toxicologically significant. Additionally, there was no evidence that erythritol has any carcinogenic, mutagenic, or teratogenic potential, and no effects on reproductive performance or fertility were reported.

A laxation threshold for erythritol cannot be clearly identified in clinical studies. In clinical studies of erythritol, some minor, transient gastrointestinal effects were observed, though only after intake of a large acute bolus (liquid) dose and/or intake on an empty stomach. The available data suggest that the threshold for laxation may be approximately 1 g erythritol/kg bw/day or more in divided doses in foods and beverages. When consumed in a bolus dose, the available data suggest that the threshold for laxation of erythritol may be in excess of 0.6 to 0.8 g erythritol/kg bw per occasion. Intakes of up to 1 g erythritol/kg bw/day in divided doses also were not associated with meaningful adverse renal effects. An intake of 1 g erythritol/kg bw/day corresponds to a daily intake of 60 g erythritol for a 60 kg adult.

The previous GRAS determinations also were supported by safety evaluations of erythritol conducted by JECFA and SCF. JECFA (2000) evaluated erythritol produced from glucose or sucrose by fermentation with either *M. pollinis* or *T. megachiliensis* and assigned it an Acceptable Daily Intake (ADI) "not specified" for use as a sweetening agent. An ADI "not specified" is applicable to a food substance of very low toxicity which, on the basis of the available data (e. g., chemical, biochemical, toxicological), the total dietary intake of the substance arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food does not, in the opinion of JECFA, represent a hazard to health. In 2003, the Scientific Committee on Food (SCF) of the European Union reviewed available data on erythritol and concluded that the use of erythritol as a food additive is acceptable. Consistent with the Committee's earlier opinion on other polyols, it was determined inappropriate to establish a numerical ADI for erythritol.

Information in the published literature since 2006 regarding the safety of intake of erythritol was identified. Additional tolerance studies of erythritol were identified, and findings from these studies continue to support the safe intake of erythritol under the GRAS conditions of use.

Yarrowia lipolytica is non-pathogenic in the generally healthy population, though recently the organism has been identified as an emerging opportunistic pathogen in compromised individuals. No reports implicating *Y. lipolytica* as a pathogen in healthy individuals were identified in searches of the literature. No evidence of toxicogenic or pathogenic potential from the use of *Y. lipolytica* in the production of food ingredients has been identified in the literature. *Yarrowia lipolytica*, strain CGMCC No. 1431, is not a GMO (Genetically Modified Organism). The specific strain of *Y. lipolytica* used in the production of erythritol is maintained using methods suitable for maintenance of yeasts used in the production of food ingredients. Following the fermentation step in the production of erythritol from *Y. lipolytica*, the fermentation solution is filtered through a membrane to remove microbial cells. The final product therefore does not contain any *Y. lipolytica*.

An analysis of the production strain *Y. lipolytica* CGMCC No. 1431, employing guidelines established to evaluate the safety of microbial enzyme preparations for use in food processing, concluded that this strain is an appropriate production organism for the production of erythritol intended for use as a component in food, because the erythritol product produced using this strain is substantially equivalent to erythritol products already determined to be GRAS (filed as GRN 76 and GRN 208)

Summary

Our summary resulting from this critical evaluation is presented below.

- Identity. The substance that is the subject of this Generally Recognized as Safe (GRAS) determination is erythritol, produced via the fermentation of glucose by *Yarrowia lipolytica*. Appropriate product specifications have been established (Food Chemicals Codex (FCC) VI, 2008) and the proposed products meet FCC specifications for food use.
- History of consumption. Erythritol has a long history of safe use as an approved flavor enhancer, formulation aid, humectant, nutritive sweetener, stabilizer and thickener, sequestrant, and texturizer.
- Intended use. The intended uses of erythritol produced from *Yarrowia lipolytica* are identical to the existing GRAS uses of erythritol produced from *M. pollinis* or *T.*

megachiliensis and are up to the specified maximum levels in the following categories of foods: bakery fillings (fruit, custard, cream, pudding) (15%); cakes and cookies (regular and dietetic) (15%); chewing gum (60%); dairy drinks (chocolate and flavored milks) (3.5%); fat-based cream used in modified fat/calorie cookies, cakes and pastries (60%); hard candies (including pressed candy, mints and cough drops) (99%); frozen dairy desserts (regular ice cream, soft serve, sorbet) (10%); puddings (instant, phosphate set) (10%); reduced- and low calorie and non-carbonated beverages (3.5%); soft candies (non-chocolate, plain chocolate, chocolate coated) (60%); sugar substitutes (carrier) (100%); and yogurt (regular and frozen) (10%).

- Estimated daily intake. The estimated average *per user* mean intake of all intended uses of erythritol is 13 g/day, and the *per user* 90th percentile intake is 30 g/day. Estimated hypothetical maximum bolus dose intakes of erythritol from beverages are 0.56 g/kg bw per occasion for young children age 2-6 years, and 0.28 g/kg bw per occasion for the U.S. population age 2 years and older.
- Safety. The safety of erythritol for use as an ingredient in food has been extensively reviewed and evaluated. The general recognition of safety for the uses of erythritol in foods was based on a critical review of data and information in the published literature. A comprehensive, critical review and summary of the biochemical, metabolic, toxicological, and clinical data was published by Munro et al (1998). The safety of erythritol for use in foods was established based on results of acute, subacute, chronic oral toxicity studies in rats, mice, and dogs; results of studies conducted to assess the potential carcinogenicity, genotoxicity, teratogenicity, and reproductive effects; absorption, distribution, metabolism, and excretion; and human tolerance. FDA was notified of the GRAS determinations for the above uses of erythritol, first in 2001 and again in 2006, and responded with no objection letters regarding the assessments. Comprehensive safety reviews of erythritol also were conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 2000) and the Scientific Committee on Food (SCF) of the European Union (SCF 2003). Findings from a review of the recently published literature continue to support the previous assessment of the safety of these uses of erythritol. The uses of erythritol remain GRAS and the intended uses of erythritol produced via the fermentation of glucose by *Yarrowia lipolytica* are therefore GRAS.

Conclusion of the Expert Panel

We, the undersigned members of the Expert Panel, have individually and collectively critically evaluated published and unpublished data and information pertinent to the safety of the intended uses of erythritol produced via the fermentation of glucose by *Yarrowia lipolytica*, and unanimously conclude that the intended uses in select foods of the proposed erythritol product, produced consistent with cGMP and meeting appropriate food-grade specifications described above, are safe.

We further conclude that the intended uses in select foods of the proposed erythritol product from *Yarrowia lipolytica* produced consistent with cGMP and meeting appropriate food grade specifications described above, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with our conclusions.

Signatures

(b) (6)

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04 May 2011
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(b) (6)

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SUBMISSION END