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May 4, 2023

Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Subject: GRAS Notification –
D-psicose As a Food Ingredient

To Whom It May Concern,

On behalf of Daesang Corporation (Daesang), we are submitting a GRAS notification for D-psicose (synonymous with D-allulose) as a food ingredient. The enclosed document provides the notice of a claim that a food ingredient, the D-psicose, described in the enclosed notification 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, as a food ingredient. We believe that this determination and notification are in compliance with Pursuant to 21 C.F.R. Part 170, subpart E.

Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,



Susan Cho, Ph.D.
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(301) 875-6454
Agent for Daesang Corporation

Daesang: D-psicose (synonymous with D-allulose)

**GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE FOR
D-PSICOSE (D-ALLULOSE)
AS A FOOD INGREDIENT**

On Behalf of Daesang Corporation
Jongno Place, 120, Changgyeonggung-ro, Jongno-gu,
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LIST OF ABBREVAITIONS

AEs = adverse events

ALP = alkaline phosphatase

ASCVD = atherosclerotic cardiovascular disease

AST = aspartate transaminase

AUC = area under the curve

BLAST = Basic Local Alignment Search Tool

bw = body weight

CAS = Chemical Abstracts Service

CEE = carbohydrate energy expenditure

CFR = Code of Federal Regulations

CFU = colony forming unit

cGMP = current Good Manufacturing Practices

COA = Certificate of Analysis

d = days

dpm = disintegrations per minute

EDI = estimated daily intake

EE = energy expenditure

EFSA = European Food Safety Authority

Expt = experiment

FDA = Food and Drug Administration

FOS = fructo-oligosaccharides

GM = genetically modified

GTP = gamma-glutamyl transpeptidase

GRAS = Generally Recognized as Safe

GRN = GRAS notice number

HACCP = Hazard Analysis and Critical Control Point

HDL-C = high-density lipoprotein cholesterol

HFD = high-fat diet

HPLC = high-performance liquid chromatography

HSD = high-sucrose diet

IL-6 = interleukin-6

IMT = intima-media thickness

LD₅₀ = mean lethal dose

LDL-C = low-density lipoprotein cholesterol

MDA = malonaldehyde

Mo = months

ND = not detected

NHANES = National Health and Nutrition Examination Survey

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NOAEL = no-observed-adverse-effect-level

NS = not specified

OECD = Organization for Economic Co-operation and Development

PAI-1 = plasminogen activator inhibitor-1

PCR = polymerase chain reaction

Pctl = percentile

PND = postnatal day

QPS = qualified presumption of safety

RH = relative humidity

RI = refractive index

RQ = respiratory quotient

SCFA= short chain fatty acid

SD = Sprague-Dawley

sdLDL =small density LDL

SPF = specific pathogen-free

TG = triglyceride

TNF- α = tumor necrosis factor alpha

US = United States

wk = weeks

w/w = weight/weight

yr = year

Daesang: D-psicose (synonymous with D-allulose)

PART 1. SIGNED STATEMENTS AND A CERTIFICATION

1.A. GRAS Notice Submission

Pursuant to 21 C.F.R. Part 170, subpart E, Daesang Corp., via its agent, AceOne RS, Inc., submits a Generally Recognized As Safe (GRAS) notice and claims that the use of D-psicose in foods, as described in Parts 2 through 7 of this GRAS notice, is not subject to the premarket approval requirements of the FD&C Act based on its conclusion that the substance is GRAS under the conditions of its intended use.

1.B. Name and Address of the Notifier

Contact person: Jason Park

Company name: Daesang Corporation

Address: Jongno Place, 120, Changgyeonggung-ro, Jongno-gu, Seoul, Korea

Telephone number: +82-2-490-5068

E Mail Address: sh_0421@daesang.com

1.C. Common or Trade Name

Common name is D-psicose, D-allulose, or pseudo-fructose.

1.D. Applicable Conditions of Use of the Notified Substance

1.D.1. Foods in Which the Substance is to be Used

Daesang proposes to use D-psicose (D-allulose) as a food ingredient in selected foods listed in Table 1: bakery products (rolls, cakes, pies, pastries, and cookies: low calorie or dietetic); low-calorie beverages; selected cereals; chewing gums; coffee mix; confections and frostings; dressings for salads; fat-based creams; frozen dairy desserts (ice cream, soft serve, and sorbet: low-calorie, reduced-calorie, and sugar-free); gelatins, pudding, and fillings (low-calorie, reduced-calorie, and sugar-free); hard candies (low-calorie); soft candies (non-chocolate, plain chocolate, and chocolate coated; low-calorie, reduced-calorie, and sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low-calorie, reduced-calorie, and sugar-free); yogurt (regular and frozen: low-calorie, reduced-calorie, and sugar-free); nutrition bars, grain-based cereal bars, and protein bars; and alcoholic beverages (premixed cocktails, wine cooler, hard seltzer, and malt beverage: low- or reduced-calorie and low-or reduced-sugar).

Daesang does not intend to use D-psicose (D-allulose) as a component of infant formula or in foods under the USDA's jurisdiction, such as meat, poultry, or egg products.

Daesang: D-psicose (synonymous with D-allulose)

1.D.2. Levels of Use in Such Foods

As shown in Table 1, Daesang proposes to use D-psicose (D-allulose) as a food ingredient (a sugar substitute/sweetener) at use levels ranging from 2 to 100%. The intended use and use levels are the cumulative intended use and use levels listed in previous GRAS notices including GRN 400, 498, 623, and 828, plus two other food categories, such as alcoholic beverages (premixed cocktails, wine cooler, hard seltzer, and malt beverage; low- or reduced calorie and low-or reduced-sugar; 3.5%), as well as nutrition bars, cereal-based bars, and protein bars (15%).

Table 1. Intended Use and Maximum Use Levels of D-psicose (D-allulose), % (w/w)

Group No	Description	Maximum use levels, % (w/w)
1	Bakery products (rolls, cakes, pies, pastries, and cookies: low calorie or dietetic)	10
2	Beverages, carbonated: low calorie	3.5
3	Beverages non-carbonated (non-alcoholic) low-calorie, reduced-calorie, sugar-free)	3.5
4	Cereals, ready-to-eat (<5% sugar)	10
5	Cereals, low-and reduced-calorie, sugar-free	5
6	Cereals, regular	2
7	Chewing gum	50
8	Coffee mix	30
9	Confections and frostings	5
10	Dressings for salads	5
11	Fat-based cream - used in modified fat/calorie cookies, cakes, pastries, and pies	10
12	Frozen dairy desserts (ice cream, soft serve, sorbet: low- calorie, reduced-calorie, sugar-free)	5
13	Gelatins, pudding, fillings (low-calorie, reduced-calorie, sugar-free)	10
14	Hard candies (low-calorie)	70
15	Soft candies (non-chocolate, plain chocolate, chocolate coated: low-calorie, reduced-calorie, sugar-free)	25
16	Jams and jellies	10
17	Sugar	10
18	Sugar substitutes	100
19	Sweet sauces and syrups (low-calorie, reduced-calorie, and sugar-free)	10
20	Yogurt (regular and frozen: low-calorie, reduced-calorie, sugar-free)	5
21	Nutrition bars, grain-based cereal bars, protein bars	15
22	Alcoholic beverages (premixed cocktails, wine cooler, hard seltzer, and malt beverage: low- or reduced calorie; low-or reduced-sugar)	3.5

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1.D.3. Purpose for Which the Substance is Used

The substance will be used as a food ingredient. In alcoholic beverages, D-psicose (D-allulose) can function as a bulking agent.

1.D.4. Description of the Population Expected to Consume the Substance

The population expected to consume the substance consists of members of the general population who consume at least one of the products described above.

1.E. Basis for the GRAS Determination: Through scientific procedures.

1.F. Availability of Information

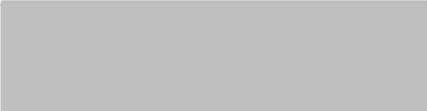
The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of Daesang or AceOne RS, Inc.

1.G. Availability of FOIA Exemption

None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552.

1.H. Certification

We certify that to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance. The notifying party certifies that the information provided herein is accurate and complete to the best of our knowledge.



Name: Jason Park

Date: 5/03/2023

Title: Director, Research and Development, Daesang Corporation

Please address correspondence to:

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AceOne RS, Inc., 5903 Hampton Forest Way, Fairfax, VA 22030

scho@aceoners.com or Susanschol@yahoo.com; 301-875-6454 (MP)

1.I. Food Safety and Inspection Service/USDA Statement

D-psicose (D-allulose) is not intended to be used in meat and/or poultry products that come under USDA's jurisdiction. Therefore, 21 CFR §170.270 does not apply.

Daesang: D-psicose (synonymous with D-allulose)

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECTS OF THE NOTIFIED SUBSTANCE

2.A. Scientific Information About the Identity of the Notified Substance

2.A.1. Scientific Information Sufficient to Identify the Biological Source

2.A.1.1. Common Name

D-psicose, D-allulose, or pseudo-fructose

Synonyms: psicose, allulose, D-altrulose, D-pseudofructose, D-erythro-hexulose, psicopyranose, D-psicopyranose, D-ribo-2-ketohexulose, psicopyranoside.

2.A.1.2. Chemical Name

D-ribo-2-ketohexose

2.A.1.3. Chemical Abstract Service (CAS) Registry Number

551-68-8

2.A.1.4. Empirical Formula

Molecular formula, C₆H₁₂O₆

2.A.1.5. Molecular Weight

180.16 g/mole

2.A.1.6. Structural Formula

Figure 1 shows the structure of D-psicose (D-allulose).

Chemical structure of D-psicose (D-allulose) is shown in Figure 1.

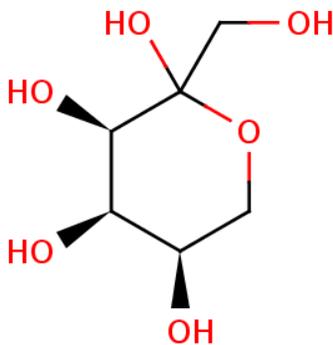


Figure 1. Chemical Structure of D-psicose (D-allulose)

Daesang: D-psicose (synonymous with D-allulose)

2.A.1.7. Background

D-psicose is a monosaccharide, an epimer of D-fructose isomerized at C-3 (Karabinos, 1952). D-psicose has 70% of the sweetness of sucrose and has a higher solubility, which makes it easy to use for food processing. Based on a plot of breath hydrogen concentration vs. calories ingested, the energy value of D-psicose is predicted to be less than 0.2 kcal/g (Iida et al., 2010). Thus, D-psicose can be categorized as a non-digestible carbohydrate. It is odorless and can be white or off-white. D-psicose is a naturally occurring monosaccharide present in small quantities in food products.

Standards of Identity

In the notice, Daesang states its intention to use D-psicose (D-allulose) in several food categories, including foods for which standards of identity exist, and are located in Title 21 of the Code of Federal Regulations. We note that an ingredient that is lawfully added to food products may be used in a standardized food only if it is permitted by the applicable standard of identity.

2.A.2. Potential Toxicants in the Source of the Notified Substance

No toxicant production is expected in the manufacture of D-psicose (D-allulose). The final product is purified through several steps during production.

2.A.3. Particle Size

The median particle size is 299.1 μm , ranging from 0.75 to 995.6 μm .

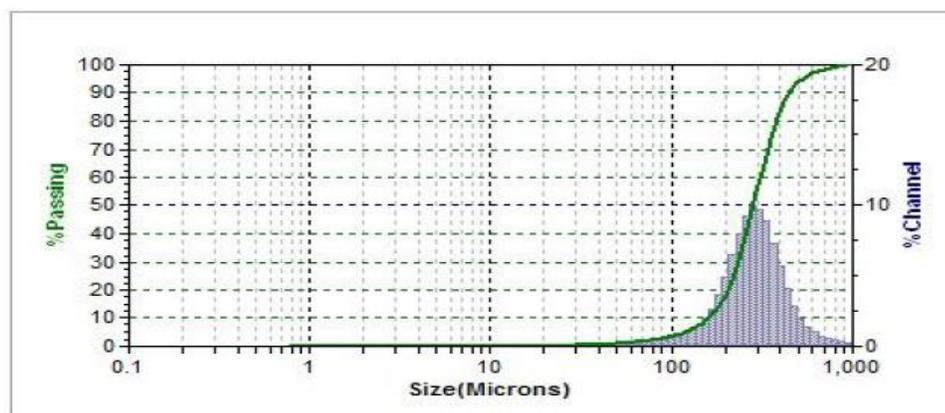


Figure 2. Particle size distribution of D-psicose (D-allulose)

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2.B. Method of Manufacture

D-psicose is manufactured from D-fructose in aqueous solution by enzymatic epimerization in the presence of calcium chloride. Immobilized *Corynebacterium glutamicum* harboring D-psicose-3-epimerase converts D-fructose to D-psicose. The enzymatic reaction involved in conversion from D-fructose to D-psicose is presented in Figure 3.

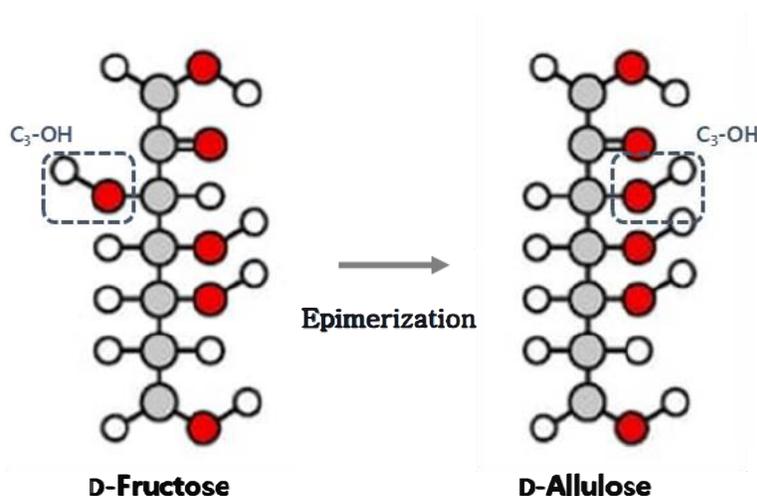


Figure 3. Conversion of D-Fructose to D-psicose (D-allulose)

Daesang's Manufacturing Process

1. Raw materials

D-allulose is manufactured by an enzymatic bioconversion process using D-fructose syrup and immobilized *Corynebacterium glutamicum* harboring D-psicose-3-epimerase originating from *Flavonifractor plautii*. Food grade D-fructose is used in the manufacturing process.

2. Sugar conversion

Fructose syrup is passed into an immobilized cell system (*Corynebacterium glutamicum* harboring D-psicose-3-epimerase) with sodium alginate gel beads in the presence of manganese sulfate or magnesium sulfate, creating D-fructose. The D-fructose then is converted to D-allulose.

3. Purification

The crude D-allulose is mixed with activated carbon to remove colored materials. D-allulose solution then is subjected to an ion exchange process with cation exchange and anion exchange resins to remove other impurities. The purified D-allulose solution is concentrated into high-purity D-allulose preparations.

4. Formulation and Standardization

Daesang: D-psicose (synonymous with D-allulose)

The final solution is formulated in two forms: syrup and crystal.

To produce D-allulose syrup, the final solution is concentrated to ≥ 63 brix ($\geq 65\%$ D-allulose, as-is basis). Using a fluid bed dryer, D-allulose syrup is subsequently dried to produce crystalline D-allulose ($\geq 98\%$ D-allulose). The absence of production microorganisms in finished ingredients is confirmed by a polymerase chain reaction (PCR) analysis.

Table 2-1 List of Raw Material

Ingredient	CAS No.	Technological Purpose
Fructose syrup	57-48-7	Starting material

Table 2-2. List of Processing Aids

Material	CAS No.	Technological Purpose
<i>Corynebacterium glutamicum</i> harboring D-psicose-3-epimerase from <i>Flavonifractor plautii</i>	Not applicable	Enzymatic conversion of D-fructose to D-psicose
Sodium alginate	9005-38-3	Immobilized agent
Calcium chloride	10043-52-4	Immobilized agent
Surfactant (Polysorbate 80)	9005-65-6	Antifoaming agent
Manganese sulfate	10034-96-5	Immobilized agent
Magnesium sulfate	7487-88-9	Immobilized agent
Activated carbon	7440-44-0	Decolorization agent
Hydrochloric acid	7647-01-0	Ion exchange agent
Sodium hydroxide	1310-73-2	Ion exchange agent

Quality assurance procedure:

Daesang observes the principles of current Good Manufacturing Practices (cGMP) and uses common food industry practices in accordance with the applicable parts of 21 CFR, part 110 of the Code of Federal Regulations. Daesang utilizes a Hazard Analysis and Critical Control Point (HACCP)-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. All processing aids used in the manufacturing process are food grade. The ion exchange resins used in the manufacturing process are food grade and comply with 21 CFR 173.25. A flow diagram of the manufacturing process is presented in Figure 4.

Daesang: D-psicose (synonymous with D-allulose)

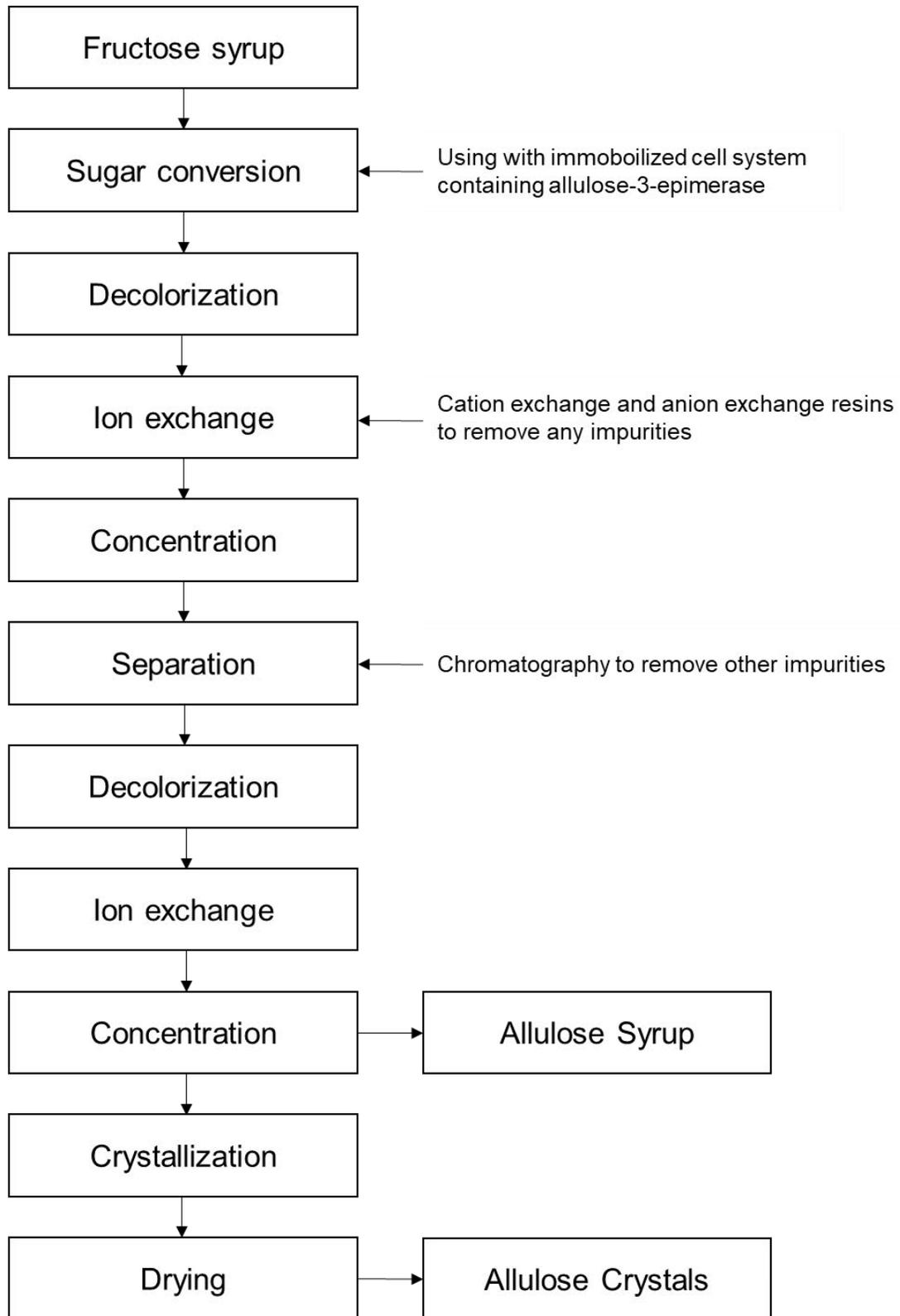


Figure 4. Manufacturing Process for D-psicose (D-allulose)

Daesang: D-psicose (synonymous with D-allulose)

2.C. Composition and Specifications of D-psicose (D-allulose)

As shown in Tables 3 and 4, the only differences in specifications are found in the concentrations of D-allulose and moisture. Specifications for microbial and heavy metal contents are the same for powder and liquid forms. As shown in Tables 5 and 6, analytical data for representative batches demonstrate that Daesang’s D-psicose (D-allulose) ingredients comply with specifications. Certificates of analysis (COA) are presented in Appendix A.

Table 3. Specifications of Daesang’s Crystalline D-psicose

Composition	D-allulose crystal	
	Specifications	Methods of Analysis
Appearance	White powder	Visual
Odor	Odorless	-
Allulose*, g/100 g	≥98.0	HPLC
Moisture, g/100 g	<5.0	AOAC 977.21
Ash, g/100 g	≤0.5	AOAC 900.02C
Pb, mg/kg	≤0.5	AOAC 2015.01
As, mg/kg	≤0.5	
Cd, mg/kg	≤0.5	
Aerobic plate counts, CFU/g	≤1,000	AOAC 990.12
Coliforms, CFU/g	<10	AOAC 991.14
Yeast, CFU/g	≤100	AOAC 997.02
Mold, CFU/g	≤100	AOAC 997.02
<i>Salmonella</i> , CFU/25 g	Negative	AOAC 999.09
<i>Staphylococcus aureus</i> , CFU/g	<10	AOAC 2001.05

*Dry weight basis.

CFU=colony forming units.

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Table 4. Specifications of Daesang's D-psicose Syrup

Composition	D-psicose syrup	
	Specifications	Methods of Analysis
Appearance	Light yellow liquid	Visual
Odor	Odorless	-
Allulose, g/100 g (dry wt. basis)	≥ 95	HPLC
Allulose, g/100 g (as-is basis)	≥ 65	HPLC
Brix	≥ 63	Brix meter
Ash, g/100 g	≤ 0.5	AOAC 900.02C
Pb, mg/kg	≤ 0.5	AOAC 2015.01
As, mg/kg	≤ 0.5	
Cd, mg/kg	≤ 0.5	
Aerobic plate counts, CFU/g	$\leq 1,000$	AOAC 990.12
Coliforms, CFU/g	< 10	AOAC 991.14
Yeast, CFU/g	≤ 100	AOAC 997.02
Mold, CFU/g	≤ 100	AOAC 997.02
<i>Salmonella</i> , CFU/25 g	Negative	AOAC 999.09
<i>Staphylococcus aureus</i> , CFU/g	< 10	AOAC 2001.05

CFU=colony forming units.

Daesang: D-psicose (synonymous with D-allulose)

Table 5. Analytical Data of Daesang's Crystalline D-psicose

Composition	Lot Number			Methods of Analysis
	220819	220823	220906	
Appearance	White powder			Visual
Odor	Odorless			-
Allulose, g/100 g, dry basis	99.6	99.8	99.7	HPLC
Moisture, g/100 g	0.62	0.62	0.65	AOAC 977.21
Ash, g/100 g	< 0.01	< 0.01	< 0.01	AOAC 900.02C
Pb, mg/kg	< 0.0035	< 0.0035	< 0.0035	AOAC 2015.01
As, mg/kg	< 0.0035	< 0.0035	< 0.0035	
Cd, mg/kg	< 0.0035	< 0.0035	< 0.0035	
Aerobic plate count, CFU/g	< 10	< 10	< 10	AOAC 990.12
Coliforms, CFU/g	< 10	< 10	< 10	AOAC 991.14
Yeast, CFU/g	< 10	< 10	< 10	AOAC 997.02
Mold, CFU/g	< 10	< 10	< 10	AOAC 997.02
<i>Salmonella</i> , CFU/25 g	Negative	Negative	Negative	AOAC 999.09
<i>Staphylococcus aureus</i> , CFU/g	< 10	< 10	< 10	AOAC 2001.05

CFU=colony forming units.

Daesang: D-psicose (synonymous with D-allulose)

Table 6. Analytical Data of Daesang's D-psicose Syrup

Composition	Lot Number			Methods of Analysis
	220814	220819	220823	
Appearance	Light yellow liquid			Visual
Odor	Odorless			-
Allulose, g/100 g, dry basis	97.9	98.1	97.9	HPLC
Allulose, g/100 g, as-is basis	67.3	71.1	68.1	HPLC
Brix	65.3	68.6	66.6	Brix meter
Moisture, g/100 g	31.8	31.5	31.6	AOAC 977.21
Ash, g/100 g	< 0.01	< 0.01	< 0.01	AOAC 900.02C
Pb, mg/kg	< 0.0035	< 0.0035	< 0.0035	AOAC 2015.01
As, mg/kg	< 0.0035	< 0.0035	< 0.0035	
Cd, mg/kg	< 0.0035	< 0.0035	< 0.0035	
Aerobic plate count, CFU/g	< 10	< 10	< 10	AOAC 990.12
Coliforms, CFU/g	< 10	< 10	< 10	AOAC 991.14
Yeast, CFU/g	< 10	< 10	< 10	AOAC 997.02
Mold, CFU/g	< 10	< 10	< 10	AOAC 997.02
<i>Salmonella</i> , CFU/25 g	Negative	Negative	Negative	AOAC 999.09
<i>Staphylococcus aureus</i> , CFU/g	< 10	< 10	< 10	AOAC 2001.05

CFU=colony forming units.

Daesang: D-psicose (synonymous with D-allulose)

Other Components Present in Daesang's D-psicose (D-allulose) Syrup

The oligosaccharides present in the final D-psicose (D-allulose) preparation are from the raw material, high fructose corn syrup. As shown in Table 7, other saccharide content goes down as the manufacturing process proceeds to the final stage. Hobbs (2009) also noted that oligosaccharides are present in starch-derived sweeteners. On a dry weight basis, typical finished D-allulose syrups are composed of $\geq 95\%$ D-psicose and small quantities of other saccharides typically found in sugar syrups (including 1.0% fructose and 0.1% glucose) (Table 7). These are common carbohydrates present in sweeteners; thus, the presence of such carbohydrates is not expected to impact the safety of D-psicose.

Table 7. Composition of Daesang's D-psicose (D-allulose)

Composition (%, dry wt. basis)		D-psicose	Fructose	Glucose	Total non-allulose saccharides
D-allulose Syrup	220814	97.9	1.0	0.1	2.1
	220819	98.1	0.8	0.1	1.9
	220823	97.9	1.0	0.1	2.1
D-allulose Crystals	220819	99.6	0.1	0.1	0.4
	220823	99.8	0.1	0.0	0.2
	220906	99.7	0.1	0.0	0.3

2.D. Identification

High performance liquid chromatography (HPLC) confirmed that the D-psicose manufactured by Daesang is chemically identical to that of the reference material (Carbosynth). The analysis confirmed the chemical equivalence of Daesang's D-psicose to commercial references as follows.

HPLC analysis

To identify and analyze the purity of D-psicose (D-allulose), syrup and crystal samples were analyzed by HPLC (Agilent 1100 series) under the following conditions:

Column: Bio-rad Carbohydrate Amine®HPX-87column, 300 mm×7.8 mm

(Catalog #125-0095 or equivalent)

Mobile phase: Deionized water 100%

Flow rate: 0.6 mL/min

Column temperature: 80°C

Detector: Refractive index (RI) detector

Similar retention times were observed for the main component (the commercial reference vs. Daesang's D-psicose syrup, Daesang's D-psicose crystal). As shown in Table 8 and Figures 5 to

Daesang: D-psicose (synonymous with D-allulose)

7, the data indicate that Daesang's D-allulose mimics or is identical to the reference (Carbosynth).

Table 8. Retention Times of D-psicose and Other Saccharides

Composition (%)	D-psicose	Fructose	Glucose	Oligosaccharides*
Retention Time (min)	20.22	12.80	9.86	6.34 - 8.16
D-allulose Crystals	20.29	12.81	9.42	6.38 - 8.05

The oligosaccharide standard used in this assay was a mixture of DP2 to DP10 (equal weights of maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoteptaose, Maltooctaose, maltononaose, and maltodecaose).

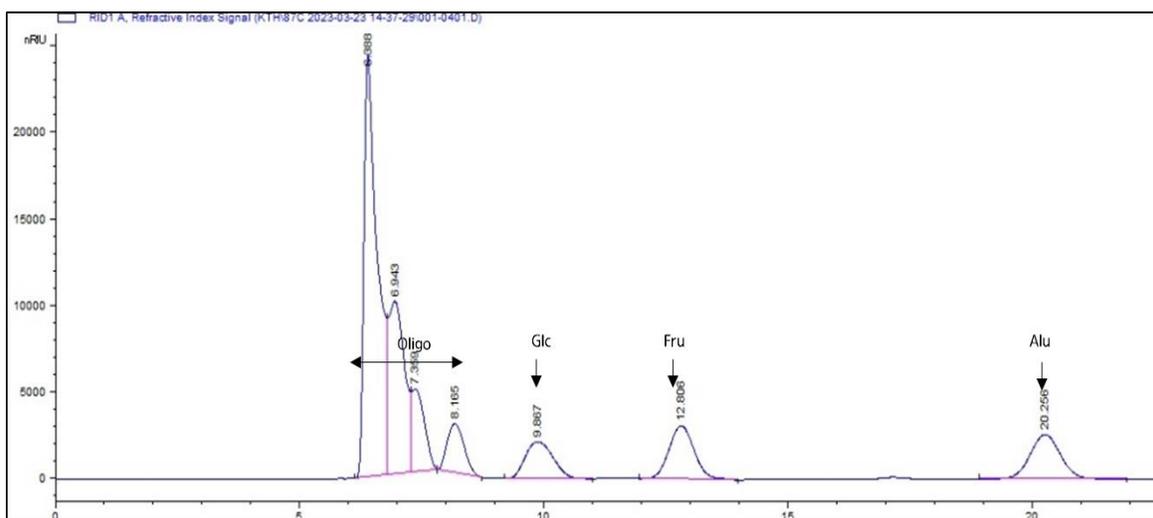


Figure 5. HPLC Chromatogram of Standards (where Alu is D-psicose)

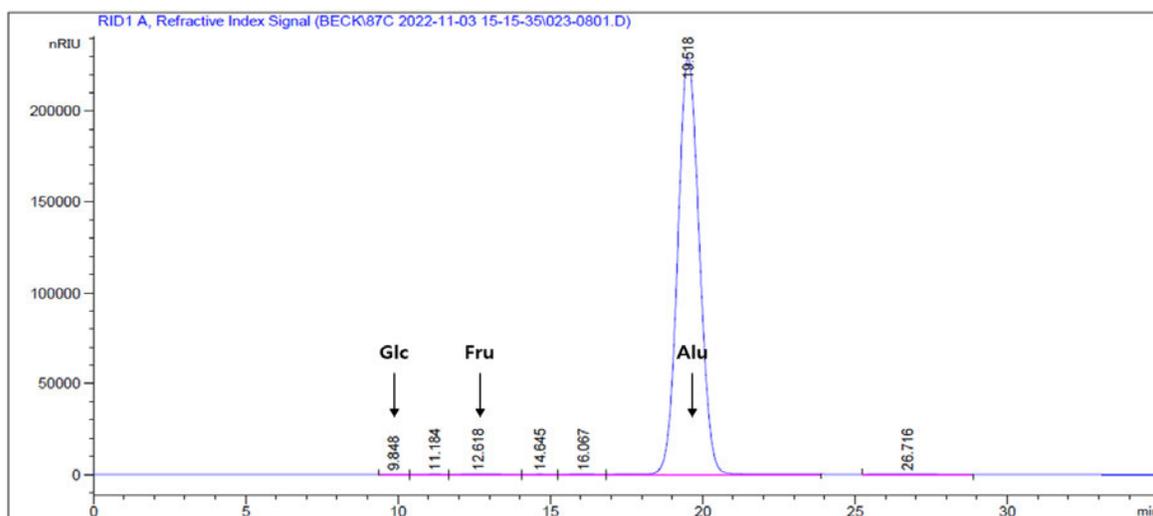


Figure 6. HPLC Chromatogram of Daesang's D-psicose (D-allulose) Crystal (where Alu is D-psicose)

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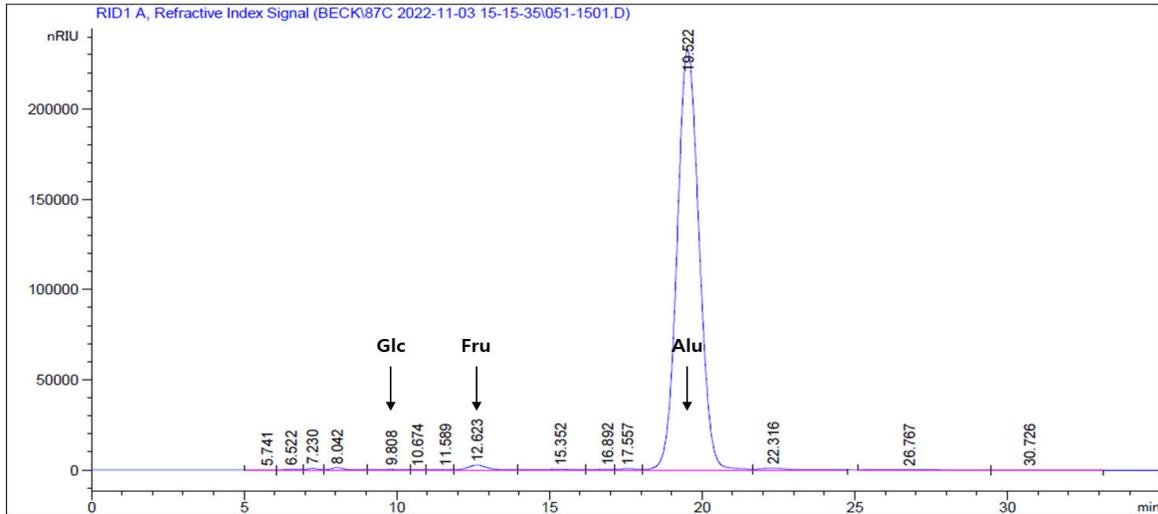


Figure 7. HPLC Chromatogram of Daesang's D-psicose (D-allulose) Syrup (where Alu is D-psicose)

Absence of Residual Production Microorganism in D-psicose (D-allulose)

No residual *C. glutamicum* DS00001, a production microorganism harboring D-psicose-3-epimerase, is present in Daesang's finished D-psicose ingredients (Appendix B).

Daesang: D-psicose (synonymous with D-allulose)

2.E. Stability of D-psicose (D-allulose) Ingredients

GRN 893 (page 15 and Appendix C of GRN 893) reported that crystalline allulose (D-psicose) was stable for up to 30 months and that liquid allulose was stable through the end of the product's shelf life for up to 9 months when stored at 4°C, 25°C, and 35°C.

Stability tests of Daesang's D-psicose crystal are ongoing under two different storage conditions. Three batches have been stored at 25°C, 65% relative humidity (RH), and 40°C, 75% RH.

As shown in Table 9-1 and 9-2, the Daesang's crystalline D-allulose was stable over the test period of four months. At 25°C and 40°C, 99.7-99.8% of D-allulose was recovered when compared to the baseline value.

It is expected that the stability of Daesang's D-psicose (crystalline and syrup forms) will be similar to those of other D-psicose preparations.

Table 9-1. Stability Results of Daesang's Crystalline D-psicose at 25°C, 65% RH*

Duration (Month)	D-psicose (% , w/w)	% Recovery	Moisture (%)
0	99.7±0.1	100.0	0.54±0.05
2	99.8±0.0	100.1	0.62±0.11
4	99.7±0.0	100.0	0.61±0.06

*Average of 3 lots; on a dry weight basis

Table 9-2. Stability Results of Daesang's Crystalline D-psicose at 40°C, 75% RH*

Duration (week)	Allulose (% , w/w)	% Recovery	Moisture (%)
0	99.7±0.1	100.0	0.54±0.05
2	99.7±0.1	100.0	0.54±0.06
4	99.8±0.1	100.1	0.59±0.05
8	99.8±0.1	100.1	0.69±0.10
12	99.8±0.1	100.1	0.52±0.04
16	99.8±0.1	100.0	0.49±0.05

*Average of 3 lots, on a dry weight basis.

Daesang: D-psicose (synonymous with D-allulose)

2.F. Intended Technical Effects

D-psicose (D-allulose) will be used as a food ingredient for low-calorie foods due to its attributes (e.g., it functions as a sweetener and a humectant).

Daesang: D-psicose (synonymous with D-allulose)

PART 3. DIETARY EXPOSURE

3.A. Exposure Estimates for D-psicose (D-allulose) Under the Intended Use

Daesang’s intended use levels are cumulative intended uses described in the previous GRAS notices (GRNs 400, 498, 693, and 838) for which the FDA had no questions on the intended use levels. In addition, two groups of new food categories are included in this GRAS determination (nutrition bar, grain-based cereal bars, protein bars; alcoholic beverages [premixed cocktails, wine cooler, hard seltzer, and malt beverage], low- or reduced-calorie; low-or reduced-sugar).

Table 10. Intended Use and Use Levels (%) from Previous GRAS Notices and the Current Notice

Group No	Description	400	498	693	828	Current notice
1	Bakery products (rolls, cakes, pies, pastries, and cookies: low-calorie or dietetic)	10	NS	10	10	10
2	Beverages, carbonated and low-calorie	2.1	3.5	3.5	NS	3.5
3	Beverages, non-carbonated (non-alcoholic): low-calorie, reduced-calorie, sugar-free)	2.1	3.5	3.5	3.5	3.5
4	Cereals, ready-to-eat (<5% sugar)	10	NS	NS	NS	10
5	Cereals, low-and reduced-calorie, sugar-free	NS	5	5	5	5
6	Cereals, regular	NS	2	2	2	2
7	Chewing gum	50	50	50	50	50
8	Coffee mix	30	NS	NS	NS	30
9	Confections and frostings	NS	5	5	5	5
10	Dressings for salads	NS	5	5	5	5
11	Fat-based cream - used in modified fat/calorie cookies, cakes, pastries, and pies	10	NS	5	5	10
12	Frozen dairy desserts (ice cream, soft serve, sorbet: low-calorie, reduced-calorie, sugar-free)	5	5	5	5	5
13	Gelatins, pudding, fillings (low-calorie, zero-calorie, reduced-calorie, sugar-free)	NS	10	10	10	10
14	Hard candies (low-calorie)	70	50	50	50	70
15	Soft candies (non-chocolate, plain chocolate, chocolate coated: low-calorie, reduced-calorie, sugar- free)	25	25	25	25	25
16	Jams and jellies	NS	10	10	10	10
17	Sugar	NS	10	10	10	10
18	Sugar substitutes	100	100	100	100	100

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19	Sweet sauces and syrups (low-calorie, reduced-calorie, sugar- free)	NS	10	10	10	10
20	Yogurt (regular and frozen; low-calorie, reduced-calorie, sugar- free)	5	5	5	5	5
21	Nutrition bar, grain-based cereal bars, protein bars	NS	NS	NS	NS	15
22	Alcoholic beverages (premixed cocktails, wine cooler, hard seltzer, and malt beverage; low-calorie or reduced-calorie; low-sugar or reduced-sugar)	NS	NS	NS	NS	3.5

EDIs Based on NHANES 2017-2018 Day 1 Survey Dataset

Using Day 1 food intake data reported in the 2017-2018 National Health and Nutrition Examination Survey (NHANES), exposure levels to D-psicose were estimated from intended uses (Tables 11-1 and 11-2). The NHANES was conducted between 2017-2018 with non-institutionalized individuals in the United States (U.S.) by the National Center for Health Statistics and the Nutrition Coordinating Center. The NHANES provides the most current food consumption data available for the American population. The food and dietary supplement record for individuals include the gram weight and nutrient data for all foods consumed during the day of the recall. All estimates were generated with USDA sampling weights to adjust for differences in representation of subpopulations. For this study 1 g is considered equivalent to 1 ml for soft drinks and formula diets for meal replacement. SAS 9.4, along with strata were used to calculate mean, 90th percentile, and standard errors (SE) for D-psicose exposure. Intakes were calculated based on the Day 1 dietary survey data.

As shown in Tables 11-1 and 11-2, approximately 75% of the population were users of D-psicose. The mean and 90th percentile all-user intakes of D-psicose were determined to be 9.1 and 21.3 g/person/day, respectively, under the intended use when the Day 1 2017-2018 NHANES dataset was used for calculation of estimated daily intakes (EDIs). Males older than 19 years of age have the highest 90th percentile intake among the various age/gender groups, with the 90th percentile value of 31.0 g/person/day in all-users. On a body weight basis, the mean and 90th percentile of all-user intakes of D-allulose were determined to be 0.13 and 0.32 g/kg bw/day, respectively. All-user children aged 2-5 years had the highest 90th percentile EDIs at 0.59 g/kg bw/day based on the Day 1 survey dataset.

Daesang: D-psicose (synonymous with D-allulose)

Table 11-1. Maximum Cumulative EDIs of D-Allulose Using Day 1 NHANES 2017-18 Survey Data, g/day*

Population	N of users	% of users	Per User (g/day)		Per Capita (g/day)	
			Mean	90 th Percentile	Mean	90 th Percentile
2+ yr	5,085	75.3	9.1±0.47	21.3±1.50	6.8±0.38	16.8±1.21
2-5 yr	415	75.9	3.6±0.37	9.5±1.27	2.7±0.32	8.3±1.04
6-12 yr	690	77.8	4.7±0.35	12.3±1.64	3.6±0.22	10.4±1.28
13-18 yr M+F	487	62.3	5.2±0.43	14.0±1.54	3.2±0.34	9.8±1.05
13-18 yr M	244	59.3	5.3±0.61	13.2±0.45	11.6±1.69	9.0±0.83
13-18 yr F	243	65.4	5.0±0.48	14.3±3.0	3.3±0.43	11.3±2.5
19-99 yr M+F	3493	76.3	10.4±0.60	25.1±1.57	7.9±0.49	20.8±1.37
19-99 yr M	1700	75.1	12.9±1.08	31.0±2.64	9.7±0.76	25.1±1.73
19-99 yr F	1793	77.4	8.0±0.62	20.3±1.73	6.2±0.52	15.3±1.41

* Assuming all foods will be used at maximum use levels.
M=male; F=female; yr=years.

Table 11-2. Maximum Cumulative EDIs of D-Allulose Using Day 1 NHANES 2017-18 Survey Data, g/kg bw/day*

Population	Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
	Mean	90 th Percentile	Mean	90 th Percentile
2+ yr	0.13±0.005	0.32±0.020	0.096±0.005	0.25±0.017
2-5 yr	0.21±0.022	0.59±0.075	0.16±0.018	0.51±0.063
6-12 yr	0.14±0.010	0.34±0.068	0.11±0.007	0.29±0.015
13-18 yr M+F	0.081±0.007	0.21±0.032	0.051±0.005	0.16±0.017
13-18 yr M	0.081±0.010	0.18±0.020	0.048±0.007	0.13±0.017
13-18 yr F	0.092±0.008	0.23±0.043	0.054±0.007	0.17±0.048
19-99 yr M+F	0.12±0.006	0.31±0.021	0.094±0.006	0.24±0.019
19-99 yr M	0.14±0.010	0.38±0.025	0.10±0.006	0.29±0.030
19-99 yr F	0.11±0.010	0.26±0.024	0.085±0.008	0.21±0.019

* Assuming all foods will be used at maximum use levels.
M=male; F=female; yr=years.

Daesang: D-psicose (synonymous with D-allulose)

EDIs Based on Averages of 2-day NHANES 2017-2018 Dataset

The mean and 90th percentile all-user intakes were 7.5 and 17.2 g/person/day, respectively, based on averages of two days of 2017-2018 NHANES dataset. Males older than 19 years of age would have the highest 90th percentile intake among the various age/gender groups, with the 90th percentile value of 27.3 g/person/day in all-users. On a body weight basis, the mean and 90th percentile all-user intakes of D-allulose were determined to be 0.11 and 0.26 g/person/day based on averages of two days' survey dataset. All-user children aged 2-5 years had the highest 90th percentile EDIs at 0.45 g/kg bw/day (based on averages of 2-day survey dataset).

The data indicate that calculation methods alone (averages of 2-day survey dataset vs. day 1 survey dataset) resulted in approximately 20-30% variations in the 90th percentile EDI values when using the same NHANES dataset.

Table 12-1. Maximum Cumulative EDIs of D-Allulose Based on Averages of 2-Day NHANES 2017-2018 Dataset, g/day *

Population	N of users	% of users	Per User (g/day)		Per Capita (g/day)	
			Mean	90 th Percentile	Mean	90 th Percentile
2+ yr	5,283	88.7	7.5±0.33	17.2±1.14	6.6±0.31	15.1±1.14
2-5 yr	414	90.4	3.1±0.31	7.6±0.97	2.8±0.30	7.3±0.97
6-12 yr	721	91.7	4.2±0.41	9.8±1.43	3.8±0.34	9.5±1.24
13-18 yr M+F	531	78.7	4.1±0.40	9.2±0.99	3.2±0.29	8.2±0.76
13-18 yr M	252	75.1	3.9±0.35	8.1±0.62	2.9±0.22	6.7±0.49
13-18 yr F	279	82.4	4.3±0.53	10.0±2.04	3.5±0.45	8.8±2.16
19-99 yr M+F	3,617	89.3	8.5±0.44	19.5±1.71	7.6±0.41	18.5±1.43
19-99 yr M	1,737	88.4	10.4±0.66	27.3±3.21	9.2±0.61	22.8±2.60
19-99 yr F	1,880	90.1	6.8±0.42	14.7±1.24	6.1±0.39	13.1±1.16

* Assuming all foods will be used at maximum use levels.

M=male; F=female; yr=years.

Daesang: D-psicose (synonymous with D-allulose)

Table 12-2. Maximum Cumulative EDIs of D-Allulose Based on Averages of 2-Day NHANES 2017-2018 Dataset, g/kg bw/day*

Population	Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
	Mean	90 th Percentile	Mean	90 th Percentile
2+ yr	0.11±0.004	0.26±0.016	0.09±0.004	0.23±0.013
2-5 yr	0.18±0.019	0.45±0.041	0.16±0.018	0.44±0.037
6-12 yr	0.12±0.010	0.32±0.038	0.11±0.009	0.30±0.036
13-18 yr M+F	0.06±0.007	0.15±0.018	0.05±0.005	0.13±0.015
13-18 yr M	0.06±0.005	0.15±0.011	0.04±0.003	0.11±0.014
13-18 yr F	0.07±0.01	0.16±0.030	0.06±0.008	0.14±0.031
19-99 yr M+F	0.10±0.005	0.24±0.016	0.09±0.004	0.22±0.015
19-99 yr M	0.11±0.006	0.26±0.023	0.1±0.005	0.26±0.023
19-99 yr F	0.09±0.006	0.21±0.019	0.08±0.006	0.19±0.013

* Assuming all foods will be used at maximum use levels.

M=male; F=female; yr=years.

Additional Variable: Impact of Using Day-1 2007-2010 NHANES dataset

In addition to calculation methods, NHANES data collection years add another variable. When NHANES 2007-2010 day 1 survey dataset was used, the mean and 90th percentile EDIs were 12.5 and 33.4 g/person/day or 0.18 and 0.46 g/kg bw, in all-users. On a body weight basis, children aged 2-5 years had the highest 90th percentile EDI at 0.59 g/kg bw/day in all-users.

Compared to the 2007-2010 NHANES dataset, employment of the most recent NHANES dataset resulted in 20-25% lower EDIs. It is noteworthy that many food codes used in GRN 400 and 623 (NHANES 2007-2010) have been dropped and are no longer available in the 2017-2018 NHANES dataset, resulting in lower EDIs when used the most recent dietary survey dataset. Details of food codes are presented in Appendix C.

Impact of Using Averages of Two-day 2007-2010 NHANES Dataset

When averages of 2 days NHANES 2017-2018 dataset were used, the mean and 90th percentile EDIs were 7.5 and 17.2 g/person/day or 0.11 and 0.26 g/kg bw/day in all-users. On a body weight basis, all-user children aged 2-5 years had the highest 90th percentile EDI at 0.45 g/kg bw/day in all-users.

When averages of 2 days NHANES 2007-2010 dataset were used, the mean and 90th percentile EDIs were 9.8 and 25.3 g/person/day or 0.14 and 0.34 g/kg bw/day in all-users. On a body weight basis, children aged 2-5 years had the highest 90th percentile EDI at 0.49 g/kg bw/day in all-users. This way of calculation alone (averages of two-day survey dataset vs. day 1 survey dataset) resulted in approximately 20-26% variation in the 90th percentile EDI values.

Daesang: D-psicose (synonymous with D-allulose)

Overall, the 90th percentile EDI values on a body weight basis did not exceed 0.5 g/kg bw/day in any age or gender groups when averages of 2-day survey datasets were used. When day 1 survey data only were used, the 90th percentile EDI values did not exceed 0.6 g/kg bw/day in any age or gender groups. These EDIs are within the safe intake levels of D-psicose.

Table 13 summarizes EDIs calculated for 2007-2010 and 2017-2018 using day 1 and averages of 2 day survey NHANES datasets.

Table 13. Summary of EDIs in All-Users Calculated for Different NHANES Years Using Day 1 and Averages of Two-day Survey Datasets

	Per User (g/person/day)		Per User (g/kg bw/day)	
	Mean	90 th Percentile	Mean	90 th Percentile
<i>U.S. 2+ y</i>				
2017-18 Day 1 only	9.1±0.47	21.3±1.50	0.13±0.005	0.32±0.020
2017-2018 2-day averages	7.5±0.33	17.2±1.14	0.11±0.004	0.26±0.016
2007-2010 Day 1 only	12.5±0.30	33.4±1.42	0.18±0.004	0.46±0.015
2007-2010 2-day averages	9.8±0.37	25.3±1.51	0.14±0.005	0.34±0.015
<i>Age 2-5 y</i>				
2017-18 Day 1 only	3.6±0.37	9.5±1.27	0.21±0.022	0.59±0.075
2017-2018 2-day averages	3.1±0.31	7.6±0.97	0.18±0.019	0.45±0.041
2007-2010 Day 1 only	4.1±0.26	9.9±0.99	0.25±0.015	0.59±0.05
2007-2010 2-day averages	3.5±0.15	8.1±0.43	0.22±0.010	0.49±0.028

These estimates are highly amplified, because D-psicose is unlikely to be used at maximum levels for all food categories under the intended use. In addition, short-term dietary surveys tend to overestimate food intake (Lambe and Kearney, 1999). Overall, intended use will result in EDIs within the safe intake range.

3.B. Food Sources of D-psicose

As shown in Table 14, D-psicose is a naturally occurring monosaccharide present in small quantities in food products, particularly in selected bakery products, sweets, and fruits (Oshima et al., 2006).

Daesang: D-psicose (synonymous with D-allulose)

Table 14. D-psicose Content in Foods

Item	mg/100 g food
Bakery products	
Sponge cake	11.0
Corn-snack	47.0
Rice cracker	27.3
Cookie	26.7
Brown sugar drop	76.5
Fried dough cake	95.6
Chocolate-chip cookie	6.4
Cereal	2.2
Dishes	
Fish broiled with soy	39.1
Simmered dishes of dried radish strips	8.1
Fermented soybeans	7.8
Seasonings and beverages	
Caramel sauce	83.0
Brown sugar	71.1
Meat sauce	15.8
Demiglace	16.3
Maple syrup	57.9
Ketchup	39.8
Worcester sauce	130.6
Coke	38.3
Coffee	0.5
Fruit juice	21.5
Tomato juice	2.4
Fruits	
Dried fig	29.6
Dried kiwi fruit	9.4
Raisins	38.7
Canned peaches	1.5
Can of mandarin oranges	8.4
Canned cherries	2.0

Adopted from Oshima et al. (2006).

Daesang: D-psicose (synonymous with D-allulose)

3.C. Estimated Daily Intakes (EDIs) of Naturally Occurring Dietary D-psicose

D-psicose levels are not listed in the USDA food composition tables or the NHANES databases for individual foods. Using the dietary content of D-psicose available from the studies of Oshima et al. (2006; Table 14), dietary EDIs were estimated. The mean and 90th percentile EDI of users are 94.8 and 260.7 mg D-psicose/person/day, respectively, as described in GRN 693. These values are comparable to the EDI value of 206 mg/person/day, which was reported by Oshima et al. (2006) by assuming a daily diet consisting of fruit, cereal, fruit juice, Bolognese spaghetti, crème caramel, coke, hamburger, and fruit cocktail.

3.D. EDI of Other Components Under the Intended Use

D-psicose syrup products contain other components such as fructose and glucose. Glucose is subject to 21CFR 184.1277 and 168.120. Fructose (high fructose corn syrup) is subject to 21CFR 184.1866. The dietary EDI of these components were not calculated.

Summary of Consumption Data

The 90th percentile EDIs of D-psicose for all users were determined to be 17-21 g/person/day (or 0.26-0.32 g/kg bw/day) based on the 2017-2018 NHANES dataset. Males older than 19 years of age would have the highest 90th percentile intake among the various age/gender groups, with the 90th percentile value of 27-31 g/person/day in all users. The 90th percentile EDI values on a body weight basis did not exceed 0.5 g/kg bw/day in any age or gender groups when averages of 2-day NHANES datasets were used. When day 1 survey data only were used, the 90th percentile EDI values did not exceed 0.6 g/kg bw/day in any age or gender groups. These EDIs are within the safe intake levels found from animal and human clinical studies.

The EDI assessments assume that Daesang's D-psicose will replace currently marketed D-psicose. Therefore, cumulative exposures are not expected to change. In addition, the EDI values presented in this notice are highly amplified estimates because D-psicose is unlikely to be used at maximum levels for all food categories under the intended use and short-term dietary surveys tend to overestimate food intake.

Daesang: D-psicose (synonymous with D-allulose)

PART 4. SELF-LIMITING LEVELS OF USE

The use of D-psicose is considered self-limiting because D-psicose attributes, such as sweetness and flavor profile, may limit its use and may impact consumer acceptability.

Daesang: D-psicose (synonymous with D-allulose)

PART 5. HISTORY OF CONSUMPTION OF THE SUBSTANCE FOR FOOD USE BY A SIGNIFICANT NUMBER OF CONSUMERS (OR ANIMALS IN THE CASE OF ANIMAL FOOD) PRIOR TO JANUARY 1, 1958

Not applicable.

Daesang: D-psicose (synonymous with D-allulose)

PART 6. NARRATIVES

6.A. Current Regulatory Status

Food products containing D-psicose are currently marketed in the USA, Japan, and Korea. In the USA, several sources of D-psicose have established GRAS status. The US FDA has issued ‘no question’ letters for a few GRAS notices (GRNs) related to food uses of D-psicose (GRN 400 submitted by CJ CheilJedang, Inc., FDA, 2012; GRN 498 submitted by Matsutani Chemical, FDA, 2014; GRNs 693 and 828 submitted by Samyang Corp, FDA, 2017 and 2020; GRN 1024 submitted by Blue California, FDA, 2023). In these GRNs, toxicity-related studies on D-psicose from the literature were presented that support the safe use of D-psicose. The FDA did not question the acceptability and suitability of these studies to establish the safety of D-psicose for the proposed food uses. The FDA did not have questions on the summary of safety, concluding that D-psicose intake of less than 0.5-0.6 g/kg bw/day is safe. Table 15 summarizes the previous GRNs and the current notice for D-psicose.

Table 15. Summary of GRAS Notices in the U.S.

GRN	Company	Production microorganism (enzyme* source)	Intended use	EDI, 90 th pctl in all users
Current notice				
Not yet available	Daesang	GM <i>Corynebacterium glutamicum</i> (harboring enzyme from <i>Flavonifractor plautii</i>)	As a sugar substitute in food applications at use levels ranging from 2 to 100% (cumulative use from GRN 400, 498, 623, and 828, plus 2 other food categories)	17-21 g/p/day or 0.26-0.32 g/kg bw/day (NHANES 2017-2018)*
Previous GRAS notices				
828	Samyang Corp.	Non-GM <i>Microbacterium foliorum</i> harboring enzyme	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	30 g/p/day or 0.42 g/kg bw/day (NHANES 2007-2010)
693	Samyang Corp.	GM <i>Corynebacterium glutamicum</i> (<i>Clostridium scindens</i>)		
498	Matsutani	GM <i>Escherichia coli</i> (K12) or <i>Streptomyces violaceoruber</i> (<i>Arthrobacter globiformis</i>)	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	24.8 g/p/day or 0.33 g/kg bw/day

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400	CJ CheilJedang	GM <i>Corynebacterium glutamicum</i> (<i>Arthrobacter globiformis</i>)	As a sugar substitute in foods at use levels ranging from 2 to 10%.	28.5 g/p/day or 0.36 g/kg bw/day
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Enzyme=D-psicose-3-epimerase; bw=body weight; GRN=Generally Recognized as Safe notice; GM=genetically modified; pctl=percentile.

g/p/d=g/person/day.

*The 90th percentile EDIs would be 25-33 g/p/d or 0.34-0.46 g/kg bw/day if the NHANES 2007-2010 dataset would be employed in exposure estimates.

The pertinent information is available as indicated below:

GRN 400: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=400>.

GRN 498: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=498>.

GRN 693: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=693>.

GRN 828: <https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=828>.

Korea

D-psicose has been approved as a food ingredient in Korea since 2016.

Japan

D-psicose has been legally marketed in Japan without separate approval processes.

Mexico

Allulose was approved as a non-caloric sweetener by the Federal Commission for the Protection against Sanitary Risk in Mexico in 2017.

6.B. Review of Safety Data

As noted above, the FDA has had no question on a few GRAS notices related to food uses of D-psicose. The FDA did not have questions on the summary of safety, concluding that D-psicose intake up to 0.5 - 0.6 g/kg bw/day is safe.

Since the specifications for D-psicose in this notice are similar to those described in previous GRAS notices, the metabolism and safety data and other pertinent information discussed in GRN 400, 498, 693, 828, and 1024 are applicable to the safety of D-psicose in this GRAS notice. The information is hereby incorporated by reference in these documents and will not be discussed in detail.

We focused our literature review on publications between January 2021 and February 2023. We excluded studies that tested a mixture of rare sugars (mixtures of D-psicose, D-tagatose, D-sorbose, etc.) as well as single-dose animal efficacy and human clinical studies,

Daesang: D-psicose (synonymous with D-allulose)

unless the studies were related to D-psicose pharmacokinetics. Findings from these newly published studies were consistent with the agency's prior decision.

6.B.1. Metabolism

6.B.1.1. Metabolic Fate of D-psicose in Animals

Since the FDA's review in 2021-2023, no new metabolism study has been published. GRN 400 (stamped page 19), GRN 693 (p 19), and 1024 (p 24-25) discussed the D-psicose metabolic pathway.

As discussed in GRN 693, following oral administration, D-psicose is partly absorbed in the digestive tract and enters the bloodstream. Maximum blood concentration (48.5 ± 15.6 $\mu\text{g/g}$) is observed at 1 hour. Excretion via urine is 20% within one hour and 33% within two hours (Tsukamoto et al., 2014). Accumulation in organs is only detected in the liver. Following intravenous administration, blood concentration of D-psicose decreases with a half-life of 57 minutes, and excretion via urine reaches almost 50% within 1 hour. Seven days after single-dose oral administration, remaining D-psicose in the whole body is less than 1%.

GRNs 400 and 693 reported that about 98% of intravenously administered D-psicose is excreted in the urine within six hours (Whistler et al., 1974). When orally ingested, urinary excretion of unchanged D-psicose ranges from 11 to 25% (Matsuo et al., 2003). The data indicate that D-psicose absorbed in the small intestine may pass into the bloodstream and be excreted in the urine without being significantly metabolized (Matsuo et al., 2003). Unabsorbed D-psicose is fermented to short chain fatty acids (SCFA) by intestinal microflora in the colon or is excreted in the feces (Matsuo, 2004).

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6.B.1.2. Metabolic Fate of D-psicose in Humans

Human studies have observed that most orally administered D-psicose is absorbed and excreted intact in urine. The small amount of D-psicose passed to the large intestine is poorly fermented and excreted in feces if not fermented. The metabolism of D-psicose appears to be similar to that of erythritol, which is quickly absorbed and excreted via urine.

A Study by Aitee et al. (2015)

Tate & Lyle commissioned a mass balance study using a radioisotope label (^{14}C) of allulose to determine absorption, excretion, and fermentability of allulose (Aitee et al., 2015; Williamson et al., 2014-abstract only). The study by Aitee et al. (2015) has not been published, but the results of the study were submitted to the FDA by Tate & Lyle (Citizen's petition) and assessed by the FDA in the context of investigating sugar label declarations of D-psicose in the U.S.

Eight healthy adult males received a single dose of a 240 mL solution containing 15 g allulose and 776 nCi of $^{14}\text{C}(\text{U})$ -allulose after a light breakfast. The test product was comprised of ~99% [$^{14}\text{C}(\text{U})$]-allulose and ~1% ($^{14}\text{C}(\text{U})$)-fructose. Blood plasma, urine, and fecal samples were collected after administration for up to 168 hours for analysis. Total plasma radioactivity recovery demonstrated that the ^{14}C radiotracer was rapidly absorbed. The average peak plasma concentration occurred within 1 hour of administration. Within 24 hours, the majority of the ^{14}C radiotracer was cleared from the plasma (Table 16). Urine and fecal samples demonstrated that urine was the major route of elimination (84-93% of recovered ^{14}C radiotracer), while feces was a minor route (2-6%). Within 48 hours, the majority of the radiotracer was eliminated via urine and feces.

Table 16. ^{14}C Radiotracer Mass Balance in Eight Humans*

Elimination Route	% of Dose Recovered
Fecal	3.12 ± 1.23
Urine	81.47 ± 13.92
Total	84.59 ± 15.14

*Adapted from Aitee et al. (2015), Tate & Lyle Citizen's Petition to the U.S. FDA.

For radioactive components, urine was the dominant route of elimination. Allulose was identified as the predominant compound in urine. Approximately 70.4% of the orally consumed allulose dose was recovered intact in the 0-48 h composite urine and feces samples. Allulose, glucose plus fructose, and unknowns accounted for 80.3%, >10%, and <7%, respectively, of the radioactivity recovered in the 24-hour human plasma composite sample.

Expired air samples for $^{14}\text{CO}_2$ detection were collected prior to and after administration at selected intervals for up to six hours. Results of the expired air recovery suggest that the D-psicose which passed to the large intestine was poorly fermented with minimal detection of ^{14}C

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in breath samples (only 6% of samples were above the limit of quantification of <50 disintegrations per minute [dpm]).

This study demonstrated that radio-labeled allulose was rapidly absorbed and urine is the major route of elimination. No adverse events were reported, and the product was well tolerated in healthy adult males.

A Study by Iida et al. (2010)

Iida et al. (2010) conducted four trials measuring breath and urine after consumption of various doses of allulose (and controls).

Energy Expenditure in Healthy Adults (Iida et al., 2010)

The first trial calculated the carbohydrate energy expenditure (CEE) and respiratory quotient (RQ) in six adult subjects after oral administration of D-psicose compared to starch hydrolysate (both at 0.35 g/kg bw) and water. Average RQ after starch hydrolysate ingestion was 0.97 while that for D-psicose or water ingestion was 0.89. After starch hydrolysate ingestion, energy expenditure increased at around 30 minutes, peaking at 60-90 minutes. On the other hand, no increase in energy expenditure was measured after D-psicose or water ingestion. The areas under the curve (AUCs) of CEE from starch hydrolysate and D-psicose ingestion were 2.33 ± 0.95 and 0.02 ± 0.62 kJ/min/kg bw, respectively. These results suggest that D-psicose is not metabolized into energy when consumed at a typical dose of 0.35 g/kg bw, corresponding to approximately 20 g for a 60 kg person in this study.

D-psicose Excretion in Urine and Fermentability in the Large Intestine (Iida et al., 2010)

The second and third studies investigated D-psicose excretion in urine and fermentability in the large intestine. Fourteen subjects received doses of D-psicose (test) or fructooligosaccharides (FOS: reference) at 0.33 g/kg bw (corresponding to approximately 20 g for a 60 kg person), 0.17 g/kg bw (10 g), or 0.08 g/kg bw (5 g). Urine was collected at 12, 24, and 48 hours (study 2). The cumulative urinary excretion rates of D-psicose (alternative name, D-allulose) after 48 hours were $66.2 \pm 12.6\%$ for 20 g, $78.6 \pm 10.6\%$ for 10 g, and $78.8 \pm 11.7\%$ for 5 g. The majority of D-psicose excreted in urine for each dose was excreted in the first 12 hours (80-82% of 48-hour cumulative excretion occurred in the first 12 hours).

Fermentability in the Large Intestine (Iida et al., 2010)

Study 3 investigated the fermentation of the D-psicose that was not absorbed in the small intestine and passed to the large intestine via hydrogen breath analysis. This study estimated an energy value for D-psicose by comparing the results against FOS, which is known to have an energy value of ~2 kcal/g (the energy value component is discussed in more detail in Iida et al., 2010). Breath hydrogen collection occurred before ingestion and hourly after ingestion for 10 hours. The AUC for excreted hydrogen gas was significantly lower for D-psicose than FOS, suggesting that minimal D-psicose is fermented in the large intestine:

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AUC: F20 = 506.9 ± 437.5 ppm/h; P20 = 56.0 ± 91.0 ppm/h

AUC: F10 = 291.0 ± 227.5 ppm/h; P10 = 26.7 ± 33.7 ppm/h

AUC: F5 = 148 ± 151.1 ppm/h; P5 = 25.3 ± 37.6 ppm/h

F5, F10, and F20 indicate FOS doses of 5, 10, and 20 g, respectively, and P5, P10, and P20 represent D-psicose doses of 5, 10, and 20 g, respectively.

Using a graph plotted for FOS based on the results (AUC), D-psicose energy values were calculated as follows: P20, 0.89 kJ/g; P10, 0.85 kJ/g; and P5, 1.61 kJ/g.

Fermentability in the Large Intestine after an Adaptation Period (Iida et al., 2010)

Study 4 investigated D-psicose fermentability in the large intestine after an adaptation period. Eight subjects ingested 5 g of D-psicose three times a day for 8 weeks. End-expiratory gas collection was conducted on the first and last day of the ingestion period. There was no statistically significant difference in breath hydrogen excretion after D-psicose ingestion before and after the adaptation period, suggesting that there is no adaptation by intestinal microflora with respect to fermentation of D-psicose.

6.B.2. Mutagenicity and Genotoxicity Studies

No new studies were identified in the literature search since GRN 828 and GRN 1024 were submitted to FDA. The mutagenicity and genotoxicity studies conducted on Daesang's D-psicose are summarized in Table 17. In addition, genotoxicity or mutagenicity studies that were reviewed in GRN 400 are summarized in Table 16. In all of the identified studies, D-psicose was reported to not produce any mutagenic or genotoxic effects.

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Table 17. Summary of *In Vitro* Mutagenicity/Genotoxicity Studies of D-psicose

Test	Concentration	Reference
D-psicose (Daesang)		
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, and <i>Escherichia coli</i> WP2urvA	0, 313, 625, 1,250, 2,500, or 5,000 µg/plate	Biotoxtech, 2022a (Appendix E)
Mammalian chromosome aberration test using Chinese hamster ovarian fibroblast cells	0, 1,250, 2,500, or 5,000 µg/mL	Biotoxtech, 2022b (Appendix E)
Micronucleus test using male Sprague-Dawley rats	0, 1,250, 2,500, or 5,000 mg/kg bw; 2 times, 24 h interval	Biotoxtech, 2022c (Appendix E)
D-psicose (CJ CheilJedang; GRN 400)		
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, and <i>Escherichia coli</i> WP2urvA (pKM101)	Up to 5,000 µg/mL	Huntington Lab, 2011 (cited in GRN 400)
Micronucleus test using CD1 mice	Up to 2,000 mg/kg/day	
Chromosomal aberration test	1,800 µg/mL	

6.B.3. Animal Toxicity Studies

Since the FDA’s last review of D-psicose in 2021-2022 (GRN 1024, U.S. FDA, 2023), no new toxicity study has been published.

The literature demonstrates D-psicose was well-tolerated in animals. Table 17 summarizes toxicity studies of Daesang’s D-psicose (An et al., 2019), and other sources of D-psicose in animals.

Acute and Subacute Toxicity Studies of D-psicose

In an acute toxicity study of Daesang’s D-psicose, 4 groups of rats were orally given D-psicose in a single dose of 0, 1.25, 2.5, or 5 g/kg bw. Rats were monitored for body weight, mortality, and clinical signs for 14 days. Organ weights and necropsy findings were examined upon sacrifice on day 15. No animals died prematurely and no treatment-related abnormalities were observed in the D-psicose group (Appendix E).

In an acute administration test (Matsuo et al., 2002), five groups of eight male Wistar rats (three weeks old) were given oral D-psicose in doses of 8, 11, 14, 17, and 20 g/kg bw. Fourteen rats that received D-psicose died within 2 days after administration: 3 rats that received 14 g/kg bw, three rats that received 17 g/kg bw, and 8 rats that received 20 g/kg bw of D-psicose. The calculated mean lethal dose (LD₅₀) values were 16.3 g/kg bw by the Behrens-Karber method and 15.8 g/kg bw by the Litchfield-Wilcoxon method. The LD₅₀ values of allulose are comparable to those of fructose (14.7 g/kg bw) and erythritol (15.3 g/kg bw). A compound that has a LD₅₀

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value of 15 g/kg bw or higher is considered ‘relatively harmless’ (Altug, 2003). D-psicose, like other monosaccharides, belongs to a group that has the lowest toxicity rating and is classified as an ordinary carbohydrate substance. Therefore, the use of D-psicose in foods and beverages is not expected to pose a safety concern.

Matsuo et al. (2002) studied the effects of sub-acute feeding of several levels of D-psicose (source, Kagawa University; purity, not specified [NS]) in rats. Male Wistar rats (3-weeks-old; 7 rats/groups) were fed diets containing 0 (control), 10, 20, 30, or 40% D-psicose for 34 days. One rat fed 30% a D-psicose diet and 5 rats fed a 40% D-psicose diet died during the experimental period. Body weight gain, food intake, and food efficiency were more extensively suppressed by the higher D-psicose diets. The weights of heart, spleen, and abdominal adipose tissue were smaller in the order of dietary D-psicose concentration. Cecal hypertrophy was observed in rats fed 10-40% D-psicose diets. Serum concentrations of glucose and triglyceride (TG) were significantly lower in the 30% D-psicose group, but liver TG concentrations were significantly higher in the 10% D-psicose group. The relative liver and kidney weights were higher in the D-psicose groups than the control group. The authors related this phenomenon to that of D-tagatose, which increased liver weight at dietary levels of 5 to 20%. They posited that unabsorbed ketohexose tends to increase liver weight. The authors did not determine the no-observed-adverse-effect-level (NOAEL) value from this study.

Subchronic Toxicity Studies of D-psicose

In the study by An et al. (2019), 80 specific pathogen-free (SPF) Sprague Dawley (SD) rats received one of following 4 treatments for 13 weeks: high-dose D-psicose (source, Samyang; purity, 98%), 5,000 mg/kg bw/day; mid-dose, 2500 mg/kg bw/day; low-dose, 1,250 mg/kg bw/day; and control, groups, 0 mg/kg bw/day.

No significant differences were found in food intake, clinical signs, and mortality among the four groups. Compared to the control group, the high-dose male group had 11.9% lower body weight. It was probably attributable to D-psicose’s role in reducing body fat by inhibiting glucose and fructose absorption (Chung et al., 2012; Matsuo et al., 2001). Therefore, body weight decreases in the high-dose males was considered a minor D–allulose-related change that was not of toxicological concern. Significant changes noted in some clinical chemistry and hematology parameters were not considered as toxicological concerns, because the values were within normal historical ranges.

Absolute and relative organ weights of the liver were significantly increased in the high-dose female group compared with the control group (high-dose vs. control: absolute liver wt., 7.74 vs. 6.51 g; relative liver wt., 2.92 vs. 2.46, $p < 0.05$). Relative liver weight in males was significantly increased in the high-dose group compared with the control group (3.26 vs. 2.84, $p < 0.05$). Absolute and relative organ weight of both kidneys in females were significantly increased

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in the high-dose group (absolute kidney wt: left, 0.92 vs. 0.78 g, right, 0.96 vs. 0.80 g; relative kidney weight: left, 0.347 vs. 0.297; right, 0.361 vs. 0.306) compared with the control group ($p < 0.05$).

Relative weight of both kidneys in males was significantly increased in the high-dose group (left, 0.371 vs. 0.317%; right, 0.377 vs. 0.325%) compared with the control group ($p < 0.05$). These results were identified as minor compound-related changes because they were within the normal range of the historical data, and these changes were not associated with any abnormalities from necropsy findings and/or histopathological examinations. No D-psicose treatment-related histopathological abnormalities were found in any organs. Thus, the NOAEL of D-psicose was determined to be 5,000 mg/kg bw/day in both male and female rats.

A sub-chronic toxicity study (3-6 months) of another source of D-psicose (manufactured by Matsutani Chemicals; purity, not specified) reported the NOAEL as 3% of the diet, which was the highest level tested (Matsuo et al., 2012).

Chronic Toxicity Study of D-psicose

Yagi and Matsuo (2009) studied the long-term toxicity of D-psicose (source, Kagawa University; purity, not specified [NS]) in 36 male Wistar rats (4-weeks old) fed diets containing 3% D-psicose (the test group: 1,280 mg/kg bw/day) or 3% sucrose (the reference group; 1,220 mg/kg bw/day) for 12 or 18 months (4 groups; 8 rats/group for 12 months and 10 rats/group for 18 months). This study did not employ multiple dose groups (it tested only one test dose level), which weakened its design.

In this study, general hematology or serum chemistry values were in the normal ranges. All values related to serum chemistry did not differ between the sucrose and D-psicose groups.

The histopathological data demonstrated that there were no toxicologically significant findings in rats given D-psicose at levels of 3% in the diet for 12-18 months. No gross pathological findings were evident at dietary doses of 3% D-psicose.

Age-related naturally occurring lesions were observed in the liver and kidneys at 12 months, but no abnormality due to ingestion of D-psicose was observed. Histopathologic observation of the liver at 18 months revealed fatty degeneration, and hepatocellular fibrosis was observed in the D-psicose group, but not in the sucrose group. These findings tended to be slight and local. Histopathological observation at 12 months showed no difference in total pathological lesions between the sucrose and the D-psicose groups (liver mean scores, 4.13 vs. 3.13; kidney, 12.3 vs. 14.1). At 18 months, the mean value for pathological lesions in the liver was significantly higher in the D-psicose group than in the sucrose group (mean scores, 2.75 vs. 3.75), but the difference was slight. At 18 months, the total value for pathological lesions in the kidneys did not differ between the sucrose and the D-psicose groups (mean scores, 14.0 vs. 14.1).

Daesang: D-psicose (synonymous with D-allulose)

Body weight gain and intra-abdominal adipose tissue weight of rats fed the D-psicose diet for 18 months were significantly lower than those in rats fed the sucrose diet. Relative liver and kidney weights were higher in the D-psicose group than in the sucrose group (D-psicose vs. control, relative tissue weights per 100 g final body weight: liver, 3.69 vs. 3.26 g at 12 mos., 3.84 vs. 3.12 g at 18 mos.; kidney, 0.74 vs. 0.63 g at 12 mos., 0.81 vs. 0.64 g at 18 mos.; all $p < 0.05$), but it was not considered to be of toxicological significance. Rather, it was due to dietary D-psicose decreasing body fat accumulation and increasing liver glycogen as a consequence of declining serum glucose and elevated serum insulin. Increased relative weight of the liver has been observed in animals fed other types of sugars, such as fructose and D-tagatose (Bär, 1999; FDA, 2011 - GRN 352). The authors concluded that administration of D-psicose at 3% in the diet (or 1,280 mg/kg bw/day) did not result in any adverse effects in rats.

Reproductive and Developmental Study of Samyang's D-psicose

Kim et al. (2019) assessed the reproductive and developmental toxicity in rats of D-psicose (source, Samyang Corp.; purity, NS). The study was a one-generation assessment of reproductive outcomes in rats, in compliance with Organization for Economic Co-operation and Development (OECD) Test Guideline 415. Prior to mating, F0 rats were dosed with 0, 500, 1,000, or 2,000 mg/kg bw/day D-psicose. Female rats were dosed continuously from two weeks before mating until day 21 of lactation. Males were dosed for 10 weeks before mating. No evidence of toxicity or mortality associated with D-psicose administration was observed. In addition, no statistically significant changes in body weight or eating behavior were observed with D-psicose administration. Male body weights trended lower in the 1,000 and 2,000 mg/kg bw/day groups, however these differences were not statistically significant.

Male rats were euthanized after the mating period, and females were euthanized after weaning their pups. Upon euthanasia, all rats underwent complete gross necropsy, and organs were preserved for histological examination. No significant changes were observed in organ weights and indexes, pre-coital time, copulation index, male fertility index, male pregnancy index, pregnancy rates, implantation, pregnancy length, gender ratios, viability indexes, lactation indexes, prenatal death rates, or number of live young at birth. No D-psicose-related manifestations were observed in necropsy and histopathological examinations.

Pups were examined for the number of live and stillborn pups (postnatal day [PND] 0). On PNDs 0, 3, 6, 9, 12, 15, 18, and 21, the body weights of the F1 pups were measured and physical findings were assessed for one random male and female in each litter. In the F1 offspring of parents receiving D-psicose, the body weights were slightly higher on days 1 to 9 but after day 9 these effects were not evident. Physical development and behavioral functions were not altered in response to D-psicose administration of the parent rats. Given the absence of any observable adverse reproductive or developmental outcomes, the study authors suggested the NOAEL of D-psicose as 2,000 mg/kg bw/day, the highest dose tested.

Daesang: D-psicose (synonymous with D-allulose)

Teratogenicity Study of D-psicose

In a prenatal developmental toxicity or teratogenicity study with D-psicose (>98% purity; Samyang Corp.), Sprague-Dawley (SD) rats were given repeated oral doses of 0, 1,250, 2,500, or 5,000 mg/kg bw/day D-psicose on gestation days 6 to 15 (4 groups; 20 rats/group; Sa et al., 2022). A solvent control group was also used. On GD 20, one day prior to the expected day of delivery, pregnant rats were weighed and anesthetized, and laparotomized to remove the uterus and its content were weighed. Fetuses were examined macroscopically for any soft tissue and skeletal changes. The evaluation indicators included general observation signs, body weight, food consumption, animal death, corpora lutea, numbers of embryonic or fetal deaths, and viable fetuses including live birth rate, fetal resorption rate, and stillbirth rate, as well as sex, body weights, and skeletal and soft tissue alterations of fetuses.

In pregnant rats, no D-psicose-related maternal toxicity, fetal malformation, or death were observed in the study. No significant differences between the control group and dose groups were found in body weight, corpus luteum number, implantation number, live birth number, fetal resorption number, litter weight, body length, and tail length. No fetus appearance malformation or visceral malformation were observed. Sternum deficiency was reported in the 2,500 and 5,000 mg/kg bw/day D-psicose groups, as well as in the solvent control group. No skeleton malformations were observed in any group. The authors concluded that D-psicose did not exhibit teratogenic effects on pregnant SD rats under the test conditions.

In addition, previous GRAS notices (GRN 828, p 24-25; GRN 1024, p 30-32) discussed subchronic toxicity studies by Nishii et al. (2017) and Matsuo et al. (2012) which found no adverse effects of D-psicose in beagle dogs and male Wistar rats. Toxicity studies in animals are summarized in Table 18.

Table 18. Summary of Toxicity Studies of D-psicose in Animals

Species	Dosage	Duration	Study type; LD ₅₀ or NOAEL	Reference
Daesang's D-psicose (D-allulose)				
Rats, SD	0, 1.25, 2.5, 5.0 g/kg bw	Single dose; observed for 14 d	Acute toxicity study; LD ₅₀ > 5.0 g/kg bw	Biototech, 2022d (Appendix E)
Other Sources of D-psicose				
Male rat	8, 11, 14, 17, and 20 g/kg	Single dose	Acute toxicity study; LD ₅₀ , 15.8-16.3 g/kg bw	Matsuo et al., 2002
Young rats	10, 20, 30, and 40% in the diet	34 d	Subacute toxicity study (feed intake, body weight gain, and organ wt); NOAEL= not determined by the author	Matsuo et al., 2002

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Dogs, beagle	0 or 200 mg/kg bw	12 wk	Subchronic toxicity study (clinical biochemistry, hematology, and body weight). NOAEL= 200 mg/kg bw/d	Nishii et al., 2017
Rats, SD	0, 1,250, 2,500, and 5,000 mg/kg bw	90 d	Subchronic toxicity study; NOAEL= 5,000 mg/kg bw/d, the highest level tested	An et al., 2019;
Male Wistar rats	3% in the diet	90 d	Subchronic toxicity study (feed intake, body weight gain, organ weights, serum biochemistry, hematology, and histology); NOAEL= 3% in diet, the highest level tested	Matsuo et al., 2012
Male rats, Wistar	3% in the diet or 1,280 mg/kg bw/d (control, 3% sucrose)	12 or 18 mo	Chronic toxicity study (feed and energy intakes, wt gain, organ wt, digestive tract size, serum biochemistry, hematology, and histology); NOAEL= 1,280 mg/kg bw/d, the highest level tested	Yagi and Matsuo, 2009
Rats, SD	0, 500, 1,000, and 2,000 mg/kg bw	Males- 10 weeks; Females- 5 weeks	Reproductive/developmental toxicity study; NOAEL= 2,000 mg/kg bw/day, the highest level tested	Kim et al., 2019
Rats, SD	0, 1,250, 2,500, and 5,000 mg/kg bw	Gestation days, 5-15	Teratogenicity study; NOAEL= 5,000 mg/kg bw/d, the highest level tested	Sa et al., 2022

NOAEL= no observed adverse effect level; bw= body weight; d=days; SD=Sprague Dawley; wk=weeks; wt= weight.

Summary of Non-clinical Safety Studies

1. D-psicose is rapidly excreted in the urine, and no significant amount of D-psicose is absorbed in the body for use as an energy source.
2. *In vitro* and *in vivo* toxicity tests, including mutagenicity and genotoxicity tests, indicate that D-psicose is not mutagenic or genotoxic.
3. Sub-chronic toxicity studies in rats showed no clinically significant treatment-related abnormalities with D-psicose in any parameters tested; food intake, body weights, blood biochemistry, hematology, and absolute and relative weights of organs. Histological examination of organs as well as urine parameters also showed no significant treatment-related abnormalities (An et al., 2019). The NOAEL was determined to be 5,000 mg/kg bw/day, the highest dose tested.
4. A 12- to 18-month chronic toxicity study of D-psicose showed that D-psicose did not cause adverse effects at the dose of 3% in the diet (or 1,280 mg/kg bw/day), the highest level tested, in rats (Yagi and Matsuo, 2009).

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5. The NOAELs found from reproductive/developmental toxicity and teratogenicity studies (Kim et al, 2019; Sa et al., 2022) were 2,000 and 5,000 mg/kg bw/day, respectively, the highest doses tested in rats.

Based on these studies, for purposes of this evaluation, a NOAEL of 5,000 mg/kg bw/day, the highest level tested, was chosen for D-psicose (An et al., 2019). D-psicose, like other monosaccharides, belongs to the group that has the lowest toxicity rating and is considered as an ordinary carbohydrate substance. Thus, the use of D-psicose in foods and beverages is not expected to pose a safety concern.

6.B.4. Animal Efficacy Studies Reporting No Adverse Effects of D-psicose

Since the FDA's last review of D-psicose (GRNs 400, 498, 693, 828, and 1024; U.S. FDA, 2012, 2014, 2017, 2020, and 2023, respectively), a few repeat-dose animal efficacy studies (Han et al., 2020; Kanasaki et al., 2021; Lee et al., 2021; Pongkan et al., 2021; Pratchay-asakul et al., 2022) were published. These studies evaluated the effects of D-psicose on various health parameters when administered at the dose level of 3-5% of diet or 0.4 g/kg bw for 4-16 weeks (Table 19). No studies reported adverse effects of D-psicose on measured outcomes. Although these studies were designed to investigate the efficacy of D-psicose on various health parameters, several safety-related endpoints were obtained during the experiments. Therefore, these studies were reviewed below as additional supporting information. Single dose studies, short-term studies (<4 weeks), or studies testing the effects of multi-components are not included in the review.

The Study by Kanasaki et al. (2021)

In a 4-week animal study by Kanasaki et al. (2021), male Wistar (Jcl: Wistar; 7 week old) rats were fed diets with or without 3% D-psicose (purity, >99%; Matsutani Chemical Industry Co., Ltd.) ad libitum for 4 weeks (N=6/group). After the feeding period, the rats fasted for 3 hours and then weighed for their final body weights. Body weight, food intake, and liver weight were recorded. Serum from the abdominal aorta blood was collected and analyzed for biochemical parameters such as serum glucose and lipid profile. Liver samples were analyzed for hepatic metabolic profile (including acylcarnitines) and metabolomics profiles (such as hierarchical cluster analysis and principal and component analysis). No adverse effects of D-psicose were noted on measured outcomes.

A Study by Pratchayasakul et al. (2022)

Pratchayasakul et al. (2022) tested the hypothesis that metformin and D-psicose (purity, NS; Matsutani Chemical Industry) equally enhance cognition and brain function in diet-induced pre-diabetic rats. Fifty-six male Wistar rats received a normal diet (N=14) or high-fat diet (HFD; N=42) to induce a pre-diabetic condition for 12 weeks. After that, 42 rats in the HFD group were randomly divided into 3 subgroups for an additional 12 weeks: HFD plus 3% D-psicose in

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drinking water (or 1.9 g/kg bw/day), HFD plus 300 mg/kg bw/day metformin in drinking water, or sterile water daily (HFD only) for 12 weeks. The normal diet-fed rats continued to consume the normal diet with sterile water daily for an additional 12 weeks (N=14/group). Measurements included fasting plasma metabolic parameters (glucose and lipid profile), brain functions (brain oxidative stress and mitochondrial function, hippocampal microglial hyperactivation, apoptosis, insulin sensitivity and synaptic dysplasticity) and cognitive function. Compared to the HFD-fed rats, D-psicose and metformin equally attenuated adverse effects induced by HFD-diet in pre-diabetic rats. No adverse effects of D-psicose were observed on measured outcomes. The authors concluded that D-psicose was safe in the presence of prediabetes.

A Study by Pongkan et al. (2021)

Using a similar design employed in Pratchayasakul et al. (2022), Pongkan et al. (2021) investigated the effects of D-psicose (source and purity, NS, but probably from Matsutani Chemical Industry Co., Ltd.) on body weight and visceral fats, indicators of glucose and lipid metabolism (fasting plasma glucose, insulin, lipid profile, cardiac function (investigated by echocardiographic study, heart rate variability, and cardiac mitochondrial function), oxidative stress (as measured by plasma and cardiac malonaldehyde [MDA]), and apoptosis in the heart of obesity-induced insulin-resistant rats (associated with chronic HFD consumption; 24 male Wistar rats, N=6/group). No adverse effects of D-psicose were observed on measured outcomes.

The Study by Lee et al. (2021)

Lee et al. (2021) evaluated if D-allulose supplementation inhibited lipid and triglyceride (TG) accumulation in HFD-fed rats. A total of 40 SD male rats (~8 week old) randomly received one of five treatments through oral gavage for 6 weeks: 1) control (chow diet, 3.5% fat); 2) 60% HFD; 3) 60% HFD (19% fat) supplemented with D-psicose powder (>98% purity; Samyang Corp.) at 0.4 g/kg bw; 4) 60% HFD supplemented with D-psicose liquid syrup (>95% purity on a dry wt. basis; Samyang Corp.) at 0.4 g/kg bw; and 5) 60% HFD supplemented with glucose at 0.4 g/kg bw. Control diet (3.5% fat) and HFD groups were gavaged with double-distilled water. Measurements included body weight gain and fat accumulation (white adipose tissue weight and adipogenic related genes) as well as blood biochemistry including glucose, lipid profile, alanine transaminase (ALT), aspartate transaminase (AST), and serum leptin and adiponectin. No adverse effects were reported on measured outcomes.

Studies by Natsume et al. (2021) and Guo et al. (2021)

Natsume et al. (2021) investigated the effects of D-allulose (purity, NS; Matsutani Chemical Industry) on insulin resistance in high-sucrose diet (HSD)-fed rats. Nineteen Wistar rats (6-weeks old) were randomly divided into 3 dietary groups for 7 weeks: HSD containing 5% cellulose, HSD containing 5% D-allulose, and a commercial diet (N=up to 5-7/group). Measurements included body weight, calorie intake, and abdominal fat as well as insulin tolerance and insulin resistance as measured by the hyper-insulinemic-euglycemic clamp at 4 and

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7 weeks. After 7 weeks, suppression of Akt phosphorylation in the soleus muscle and epididymal fat tissues (N =4-5/group), and plasma adipocytokine levels (N =5-7) were measured. No adverse effects of D-psicose were reported on measured outcomes.

Guo et al. (2021) investigated the effect of D-allulose on HFD-induced insulin resistance using the hyperinsulinemic–euglycemic-clamp method and intramuscular signaling analysis. Eighteen Wistar rats (8 weeks old) were randomly divided into three dietary groups: chow diet, HFD with 5% cellulose, and HFD with 5% D-psicose (purity, NS; Matsutani Chemical Industry). After 4 weeks of feeding, body weight, body fat composition, glucose metabolism and insulin sensitivity (fasting blood glucose and fasting plasma insulin, glucose levels during insulin tolerance test and intraperitoneal glucose tolerance test, and hyperinsulinemic-euglycemic clamp insulin resistance test), the plasma concentrations of leptin, adiponectin, and tumor necrosis factor alpha (TNF- α), and cell signaling pathway components in the skeletal muscle were measured. No adverse effects of D-psicose on measured outcomes were observed.

The Study by Han et al. (2020)

A total of 40 male C57BL/6J mice (5 weeks old) were randomly divided into four groups (n = 9) and fed one of 4 experimental diets for 16 weeks: normal diet control (American Institute of Nutrition AIN-76 semi-synthetic diet); high-fat diet control (HFD, 20% fat plus 1% cholesterol based on the AIN-76 diet), 5% erythritol in HFD (5% erythritol substituted for sucrose in HFD, w/w), or 5% D-psicose in HFD (5% D-psicose substituted for sucrose in HFD, w/w; D-psicose source, Sigma–Aldrich, purity, NS). Measurement outcomes included plasma lipid profile and adipokines (leptin, resistin, and adiponectin), energy expenditure (EE) and whole-body oxygen consumption, histological examination of liver and epididymal white adipose tissue, fecal short chain fatty acids concentrations, and fecal microbiota. No adverse effects were reported on measured outcomes.

Previous GRAS notices (GRN 400 - pages 15-17; GRN 498 - pages 12-14; GRN 693 - pages 23-27; GRN 828 - pages 26-29; GRN 1024 – pages 34-41) reported that several animal studies found no adverse effects of D-psicose. These animal studies showed that D-psicose at the level of 3-5% in the diet did not cause any adverse effects (Baek et al., 2010; Chung et al., 2012; Hossain et al., 2012; Itoh et al., 2015; Kanasaki et al., 2019; Kim et al., 2017; Matsuo and Izumori, 2004, 2006; Matsuo et al., 2001a, 2001b; Nagata et al., 2015; Ochiai et al., 2013, 2014).

It is noteworthy that a study by Han et al. (2016) compared the effects of various sugar substitutes on organ weights and lipid metabolism indicators in male C57BL/6J mice. These mice were fed a HFD with or without various sugar substitutes (5% D-glucose, D-fructose, erythritol, or D-psicose; n = 10 per group) for 16 weeks. Normal fat diet and HFD alone served as controls (a total of 6 groups). Body weight was markedly lower in mice fed HFD supplemented with D-psicose compared to the HFD control group and the HFD with other

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monosaccharides groups from week 8. Organ weights were measured to determine whether this was the cause of body weight reduction. Muscle and kidney weight were significantly higher in the HFD control group than in the normal fat diet group. Weights of muscle and kidney in the D-psicose group were not significantly different from those of the normal fat diet group, but were significantly lower than other HFD groups, including the erythritol, glucose, and fructose groups. The liver weight of the D-psicose group was significantly lower than the HFD group, while other HFD groups were not statistically different than that of the HFD group (normal fat vs. HFD vs. allulose+HFD vs. erythritol+HFD vs. glucose+HFD vs. fructose+HFD; 3.41 ± 0.05 vs. 5.49 ± 0.32^a vs. 4.35 ± 0.08^b vs. 5.37 ± 0.25^a vs. 5.22 ± 0.15^a vs. 5.68 ± 0.35^a ; ^{a,b}mean values with different superscript letters were significantly different among HFD-fed groups, $P < 0.05$). In summary, D-psicose only attenuated the liver and kidney enlargements induced by HFD, while glucose, fructose, and erythritol did not show such effects. There was no evidence of liver and kidney enlargement effects of D-psicose in this 16-week study with young male C57BL/6J mice.

None of the animal efficacy studies reported adverse effects of D-psicose. For these ‘pivotal’ studies, the dose levels represent the maximum doses administered, rather than absolute safety endpoints.

Table 19. Recently Published Animal Efficacy Studies Reporting No Adverse Effects of D-psicose

Species	Dosage	Length	Measurement endpoints	Reference
12 Male Jcl: Wistar rats (7 wk old; N = 6/group)	0 or 3% D-psicose diet	4 wk	Body wt.; food intake; serum biochemistry (glucose and lipid profile); hepatic metabolic profile (including acylcarnitines) and metabolomics profiles	Kanasaki et al., 2021
24 Male Wistar rats (age, NS)	2 groups initially: normal fat diet or HFD for 12 wk; Afterwards, 4 groups: HFD groups were divided into 3 groups for additional 12 wk; HFD, HFD + 3% D-psicose (or 1.9 g/kg bw/day), or HFD + 300 mg/kg bw/day metformin. D-psicose (purity, NS; Matsutani Chemical)	12 wk	Body wt. and visceral fats; metabolic parameters; peripheral insulin resistance; cardiac functions (heart rate variability and hepatic mitochondrial function and apoptosis) in obesity-induced insulin-resistant rats	Pongkan et al., 2021
56 Male Wistar rats (age, NS)	HFD + 300 mg/kg bw/day metformin. D-psicose (purity, NS; Matsutani Chemical)	12 wk	Fasting plasma metabolic parameters; brain and cognitive functions in pre-diabetic rats	Pratchayasakul et al., 2022

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40 SD rats (maybe 9 wk old)	5 groups: Control (3.5% fat); 60% HFD control; 60% HFD (19% fat) supplemented with D-psicose powder (>98%), D-psicose liquid syrup (>95% on a dry wt. basis) or glucose at 0.4 g/kg bw by oral gavage (D-psicose from Samyang Corp.)	6 wk	Body wt. gain and fat accumulation; blood biochemistry, including glucose, lipid profile, ALT, AST, and serum leptin and adiponectin	Lee et al., 2021
19 Wistar rats (6 wk old), HSD-induced insulin resistance model	HSD containing 5% cellulose, HSD containing 5% D-allulose, or a commercial diet	7 wk	Body wt.; calorie intake; abdominal fat; insulin tolerance and insulin resistance at 4 and 7 weeks; after 7 weeks, suppression of Akt phosphorylation in the soleus muscle and epididymal fat tissues, and plasma adipocytokine levels	Natsume et al., 2021
18 Wistar rats (8 wk old)	3 groups: chow diet, HFD with 5% cellulose, and HFD with 5% d-allulose	4 wk	Body wt.; body fat composition; glucose metabolism and insulin sensitivity; plasma concentrations of leptin, adiponectin, and tumor necrosis factor (TNF)- α ; cell signaling pathway components in the skeletal muscle	Guo et al., 2021
36 Male C57BL/6J mice (5 wk old)	Normal fat diet; HFD* control; HFD+5% erythritol; HFD+5% D-psicose (5% erythritol or D-psicose substituted for sucrose in HFD)	16 wk	Plasma lipid profile and adipokines; energy expenditure and whole-body oxygen consumption; histological examination of liver and epididymal white adipose tissue, spleen, and muscle weight; fecal short chain fatty acids and microbiota	Han et al., 2020

*In a study by Han et al. (2020), HFD contained 20% fat plus 1% cholesterol based on the AIN-76 diet. ALT=alanine aminotransferase; AST=aspartate aminotransferase; bw=body weight; HFD=high-fat diet; HSD=high-sucrose diet; NS=not specified; SD=Sprague Dawley; wk=weeks.

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6.B.5. Human Clinical Studies

GRN 1024 (pages 42-48), GRN 828 (pages 29-31), GRNs 693 (page 27), 498 (page 14), and 400 (pages 20-21) reported that several repeated human clinical studies found no adverse effects of D-psicose on glucose and lipid metabolism indicators as well as clinical chemistry parameters that included liver and renal function indicators and/or hematology. Due to substantial equivalence of the specifications and composition between Daesang's D-psicose and those discussed in previous GRAS notices, the information and data discussed in those GRAS notices are pertinent to the evaluation of the safety of the Daesang's D-psicose in this GRAS determination. Therefore, this notice incorporates, by reference, the safety and metabolic studies discussed in the previous GRAS notices, but will not discuss previously reviewed references in detail.

Daesang has also updated the literature on D-psicose published until February 2023. Any pertinent articles are discussed and referenced below. The safety of D-psicose is supported by an extensive number of clinical studies, many of which address the primary safety issues regarding D-psicose. A potential side effect of non-digestible carbohydrates is gastrointestinal symptoms. Thus, effects of D-psicose on gastrointestinal symptoms are included in this review.

6.B.5.1. Gastrointestinal Tolerance Studies in Humans

A Study by Iida et al. (2007; written in Japanese with English abstract)

Iida et al. (2007) reported that D-psicose intakes of up to 0.6 g/kg bw was well-tolerated in five healthy male and five healthy female subjects, aged 20-30 years. D-psicose (>98% purity) was provided from Rare Sugar Research Center of Kagawa University, Japan. All subjects ingested 0.4 g/kg bw of D-psicose for the first dose. Those with severe diarrhea were excluded from the test group for the next test procedure. For those without diarrhea, the dosage of D-psicose was increased by 0.1 g each time up to 0.9 g/kg bw with a week of washout between treatments. The time of intake was principally set to 10 AM with a one week of washout period, making a 1-week intake frequency. Stool characteristics and gastrointestinal symptoms were evaluated. No diarrhea was reported when the subjects consumed D-psicose at levels of 0.4-0.5 g/kg bw. When the dosage was escalated to 0.6 g/kg bw, diarrhea occurred in one male. At the level of 0.7 g/kg bw, two females experienced diarrhea. Two males and three females had diarrhea with the 0.8 g/kg bw dose. At the 0.9 g/kg bw dose, two males did not suffer from diarrhea. The authors concluded that the maximum non-effect level of D-psicose in causing diarrhea was estimated as 0.5 g/kg bw for males, 0.6 g/kg bw for females, and 0.55 g/kg bw for the mean value. These levels may correspond to 35-42 g for an adult weighing 70 kg. This study was described in detail in GRN 400 (page 21).

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A Study by Han et al. (2018a)

Han et al. (2018a) conducted two separate open-label experiments to assess gastrointestinal tolerance of D-psicose (purity, NS; CJ CheilJedang, Inc.) in 30 healthy young adults (21-30 yr).

Experiment (Expt) 1 aimed to determine the maximum single dose for occasional ingestion. The ingestion of allulose started with 0.1 g/kg bw for the first week, 0.2 g/kg bw for the third week, 0.3 g/kg bw for the fifth week, 0.4 g/kg bw for the seventh week, 0.5 g/kg bw for the ninth week, and 0.6 bw for the eleventh week. A wash-out period of one week was allowed every other week. One female subject dropped Experiment 1 due to illnesses unrelated to the treatment. When the dose of D-psicose was gradually increased in steps of 0.1 g/kg bw to identify the maximum single dose for occasional ingestion in 30 young healthy adults, no cases of severe diarrhea or gastrointestinal symptoms were noted until a dose of 0.4 g/kg bw was reached. Severe symptoms of diarrhea were noted at a dose of 0.5 g/kg bw.

In Expt 2, the maximum total daily intake of D-allulose for regular ingestion was investigated. The 19 individuals who participated in Experiment 2 visited the study center every day, with no wash-out periods. D-allulose, divided into 1 to 4 doses, was consumed throughout the day. The study was terminated when grade 3 diarrhea or gastrointestinal symptoms were reported. Accordingly, the dose administered right before the termination was regarded as the maximum daily total intake of D-allulose for regular ingestion. Increasing the total daily D-psicose intake gradually to 1.0 g/kg bw for regular ingestion resulted in incidences of severe nausea, abdominal pain, headache, anorexia, and diarrheal symptoms.

Based on these results, the maximum single dose and maximum total daily intake levels of D-psicose were determined to be 0.4 g/kg bw/single dose and 0.9 g/kg bw/day, respectively. These maximum tolerable levels of D-psicose are similar to that of erythritol (0.66 g/kg bw/day or 45 g/person/day). Studies evaluating the effects of D-psicose on gastrointestinal tolerance are summarized in Table 20.

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Table 20. Summary of Studies Evaluating the Effects of D-psicose on Gastrointestinal Tolerance and Safety in Humans

Subjects	Daily dosage	Length	Measurements	Results	Reference
10 Healthy males and females	Up to 0.9 g/kg bw/d	Acute exposure with a week of washout between treatments	Gastrointestinal symptoms; physical conditions	No gastrointestinal symptoms up to 0.5 g/kg bw/d for males and 0.6 g/kg bw/d for females	Iida et al., 2007
19-30 Healthy males and females	Gradually increased in steps of 0.1 g/kg bw	Expt 1, acute exposure with a week of washout between treatments; Expt 2, dose escalating study, 8 days	Gastrointestinal symptoms; tolerance grade	Expt 1, maximum single dose, 0.4 g/kg bw; Expt 2, maximum total daily intake, 0.9 g/kg bw/day	Han et al., 2018a

bw=body weight; d=day; Expt=experiment; wk=weeks.

6.B.5.2. Human Clinical Studies Evaluating Other Safety Parameters

Short-term studies (mostly related to glycemic responses) are not included in this review because these single-dose studies cannot accurately predict the safety (related to metabolic parameters) associated with long-term use of D-psicose.

Table 21 summarizes longer term studies evaluating the effects of D-psicose on glucose, lipid, and energy metabolism, body composition, and renal and hepatic function indicators in humans (Han et al., 2018b; Hayashi et al., 2010; Tanaka et al., 2020, 2021). The studies by Han et al. (2018b), Hayashi et al. (2010), and Tanaka et al. (2020) were discussed in detail in GRN 1024 (pages 42-48), so they will not be discussed in detail in this GRAS determination. It is noteworthy that consumption of D-psicose at daily doses up to 15 g for 48 weeks did not result in adverse effects on risk factors for atherosclerotic cardiovascular disease, glucose metabolism indicators, liver function indicator enzymes, fatty liver scores, renal function indicators, and the incidence of adverse events (AEs) (Tanaka et al., 2020).

The study by Tanaka et al. (2021) evaluated the long-term consumption effects of D-psicose on lipid profile, risk factors, and markers of atherosclerotic cardiovascular disease (ASCVD), and biliary enzyme levels in 20 patients with hypercholesterolemia (low-density lipoprotein cholesterol [LDL-C] conc., <160 mg/dL) under statin therapy (aged 20-64 years;

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Japan). Subjects consumed either 15 g/day D-psicose or erythritol (placebo), divided into three doses, for 48 weeks with a follow up at week 52. Clinical examinations were performed every eight weeks, from initial consumption until week 52. Eighteen subjects completed the study. No significant effects of D-allulose were observed in blood lipid profile, risk factors of ASCVD (including: small-dense LDL [sdLDL], TNL-alpha, MDA-modified LDL [MDA-LDL], interleukin-6 [IL-6], and plasminogen activator inhibitor-1 [PAI-1]), maximum carotid intima-media thickness (IMT), biliary gamma-glutamyl transpeptidase (GTP) and ALP levels, clinical chemistry, hematology, and urinalysis parameters, blood pressure, and AEs. The exception was a statistically significant decrease in blood high-density lipoprotein cholesterol (HDL-C) concentrations (HDL-C; week 0 vs. week 8, week 40, week 48 vs week 52: 69.6 vs. 59.6 vs. 60.4 vs. 62.9 vs. 67.9 mg/dL; $P < 0.05$ for all datapoints except week 52). However, HDL-C concentrations stayed within the standard range during the intervention period and gradually increased after week 32. No significant differences in the incidence of AEs were found between the D-psicose and the erythritol groups. The authors concluded that a long-term consumption of D-allulose did not result in clinically significant changes in measured outcomes.

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Table 21. Summary of Human Clinical Studies Reporting No Adverse Effects of D-psicose*

Subjects	Daily dosage	Length	Measurements	Results	Reference
20 patients with hypercholesterolemia under statin therapy, 20-64 y, (18 completed the study)	15 g/d D-psicose (test) or erythritol (placebo)	48 wk-intervention, follow-up at wk 52	Lipid profile; risk factors for atherosclerotic cardiovascular disease (ASCVD including sdLDL, TNF-alpha, MDA-LDL, IL-6, maximum carotid IMT, and PAI-1), biliary enzymes (gamma-GTP and ALP); clinical chemistry, urinalysis, and hematology parameters; blood pressure; AEs	No clinically significant adverse effects of D-psicose were observed on measured outcomes despite decreased blood HDL-C conc. (HDL-C stayed within normal ranges during the study)	Tanaka et al., 2021
90 adults, 20-65 y, borderline diabetes plus high LDL-C conc. (82 completed the study)	0, 5, or 15 g/d in beverage (purity, NS)	48 wk-intervention, follow-up at wk 52	Risk factors for ASCVD; glucose metabolism indicators; liver function indicator enzymes; fatty liver scores; renal function indicators; AEs	No adverse effects were observed on measured outcomes	Tanaka et al., 2020
144 healthy normal or overweight, 20-40 y (121 subjects completed the study)	0, 8, or 14 g in beverage (divided into 2 doses; purity, NS)	12 wk	Body composition; nutrient intake; plasma glucose and lipid metabolism indicators; clinical chemistry including liver/renal function indicators and inflammation biomarkers	No adverse effects were observed on measured outcomes	Han et al., 2018b
17 Normal subjects, 29-40 y, fasting blood glucose, <110 mg/dL	0 or 15 g/d (divided into 3 doses; >98.0% purity)	12 wk with 4 wk follow-up	Gastrointestinal symptoms; hematology; clinical chemistry including indicators of glucose/lipid metabolism and liver/renal functions; urine parameters; AEs	No adverse effects on measured outcomes; no significant differences in incidence of AEs between treatments	Hayashi et al., 2010

*Excluded single dose studies; High-LDL-C is defined as 120-159 mg/dL in Tanaka et al. (2020).

Daesang: D-psicose (synonymous with D-allulose)

AEs=adverse events; d=days; wk= week; y=years; ALP=alkaline phosphatase; GTP=gamma-glutamyl transpeptidase; HDL-C=high-density lipoprotein cholesterol; IL-6=interleukin-6; IMT=intima-media thickness; MDA-LDL=malonaldehyde modified LDL; NS=not specified; PAI-1=plasminogen activator inhibitor-1; sdLDL=small-dense LDL; TNF- α =tumor necrosis factor-alpha.

Daesang: D-psicose (synonymous with D-allulose)

Conclusion:

From human tolerance studies of D-psicose, Han et al. (2018a) concluded that single doses up to 0.4 g/kg bw or total daily doses up to 0.9 g/kg bw/day were well-tolerated in adults. On the other hand, Ida et al. (2007) concluded that the maximum non-effect level of D-psicose in causing diarrhea was estimated as 0.5 g/kg bw for males, 0.6 g/kg bw for females, and 0.55 g/kg bw for the mean value. These levels may correspond to 35-49 g for an adult weighing 70 kg. Long-term use (12-48 weeks) of D-psicose at daily doses of up to 15 g did not result in any adverse effects on glucose and lipid metabolism indicators, liver and kidney function indicators, or incidence of AEs in humans.

6.B.6. Allergenicity Considerations

There is no residual production microorganism in the D-psicose (D-allulose) preparation. In addition, total protein content is less than 0.02%. It is not likely that D-psicose (D-allulose) causes allergenic reactions.

6.B.7. Safety of Production Organism

Safety of Production Microorganism

Daesang's production microorganism was derived from *Corynebacterium glutamiicum* ATCC 13032, which was obtained from the U.S. Bank of Strains. *C. glutamiicum* is a Gram-positive, non-pathogenic bacterium that is distributed in the soil and is more valuable as a microorganism that is safe for humans. *C. glutamicum* is widely used in the production of various food ingredients, such as L-leucine, D-tagatose, D-psicose, L-lysine, etc. The *C. glutamicum* species is regarded as having a qualified presumption of safety (QPS) when used as a production organism for amino acids and other food or feed ingredients (European Food Safety Authority [EFSA], 2021). Genetically modified *C. glutamiicum* ATCC 13032 has also been safely used as a production microorganism in the production of 2'-fucosyllactose (2'-FL, GRN 932, FDA, 2021).

Safety of Enzyme

D-psicose-3-epimerase is composed of 294 amino acids and its amino acid sequence is presented below:

```
1   MNPIGMHYGF WSHNWDEIAY IPLMEKLAWL GFDICEVASA EWGYDDARL RELKACADHN
61  GLGITYSIGL EAKYDLASDD PAVRENGIRH VTRILEMPK VGAAILNGVS YAGWQALPDH
121 GITLDEKRRK EELALESMSR LMKVAEDCGV LYCCEVVNRF EQYLLNTAKE GVEFVKRLGS
181 PNA RVLLDTF HMNIEEDSMV DAILEAGPWL GHFHVGNNR RPAGSTNRLP WKDMAAALKQ
241 VNYQGAIVME PFVLMGGTIP YDIKVRDLS GGAGEAGLDE MAGRACRFLK ELTA-
```

D-psicose-3-epimerase did not show any homology with those of known allergenic and toxicogenic proteins when they were screened using <http://allergenonline.org/databasefasta.shtml>

Daesang: D-psicose (synonymous with D-allulose)

and the Basic Local Alignment Search Tool for Protein (BLAST) version 2.7.0 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>; accessed on September 6, 2017), respectively.

6.C. Potential Adverse Effects

Like dietary fiber and other non-digestible carbohydrates that are not absorbed in the upper gastrointestinal tract, D-psicose may cause gastrointestinal symptoms such as nausea and diarrhea when consumed in large quantities.

Daesang: D-psicose (synonymous with D-allulose)

6.D. Safety Determination

Numerous human and animal studies have reported no major adverse effects. Daesang Corp. uses an HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications. There is broad-based and widely disseminated knowledge regarding the chemistry of D-allulose. This GRAS determination is based on generally available data and information and widely accepted opinion about the safety of D-allulose. The literature indicates that D-allulose consumption does not result in adverse effects for various health outcomes.

The following safety evaluation fully considers the composition, intake, nutritional, microbiological, and toxicological properties of D-allulose, as well as appropriate corroborative data.

1. Analytical data from multiple lots indicate that D-allulose complies reliably with the established food-grade product specifications.
2. Daesang's D-allulose will be used as a food ingredient at use levels ranging from 2 to 100% as a food ingredient in selected low-calorie, reduced-calorie, or sugar-free foods as listed in Table 1.
3. The LD₅₀ value of D-allulose in rats is 15.8-16.3 g/kg. A chronic toxicity study (12-18 months) in rats showed that D-allulose at a dose of 1,280 mg/kg bw/day, the maximum level tested, did not show adverse effects. A 90 day subchronic toxicity study in rats reported the NOAEL for D-allulose as 5,000 mg/kg bw/day, the highest level tested in rats. A 90 day subchronic toxicity study in beagle dogs found that 200 mg/kg bw/day, the highest level tested, was well-tolerated with no side effects.
4. A human clinical study showed that the maximum single dose and daily tolerable levels in humans were 0.4 and 0.9 g/kg bw/day, respectively.
5. The proposed food use results in EDIs that are within the safe intake levels found from animal and human clinical studies. The EDI assessments are based on the assumption that Daesang's D-allulose will replace currently marketed D-allulose. Thus, cumulative exposures are not expected to change. In addition, the EDIs presented in this notice are highly amplified estimates.
6. In previous GRAS notices (GRNs 400, 498, 693, 828 and 1024) to the FDA, the safety of D-allulose has been established in animal toxicity and mutagenicity studies, and is further supported by human clinical studies.

Daesang: D-psicose (synonymous with D-allulose)

7. Safety of *C. glutamicum* has been proven as a commonly used production microorganism for various food ingredients. In addition, there is no residual production microorganism in finished D-psicose ingredients.

8. The protein content of D-psicose is less than 0.02% in finished D-psicose ingredients. It is not likely that D-psicose will cause allergenic reactions.

Daesang: D-psicose (synonymous with D-allulose)

6.E. Conclusions and General Recognition of the Safety of D-psicose (D-allulose)

6.E.1. Common Knowledge Element of the GRAS Determination

D-allulose has been safely used as a food ingredient around the world for a decade. A number of comprehensive reviews of the safety of D-allulose have been published (Braunstein et al., 2020; Hossain et al., 2015b). In addition, the FDA has not questioned the safety of D-allulose (GRN 400, FDA 2012; GRN 498, FDA, 2014; GRN 693, FDA, 2017; GRN 828, FDA, 2020; GRN 1024, FDA, 2023), satisfying the “common knowledge” element of GRAS determination.

6.E.2. Technical Element of the GRAS Determination (Safety Determination)

Numerous human and animal studies have reported benefits of D-allulose with no major adverse effects. Daesang uses a HACCP-controlled manufacturing process and rigorously tests its final production batches to verify adherence to quality control specifications, standards consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food grade and/or commonly used in fermentation and food manufacturing processes.

There is broad-based and widely disseminated knowledge concerning the chemistry of D-allulose. The literature indicates that D-allulose has no adverse effects. In addition, the intended uses of D-allulose have been determined to be safe through scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the “technical” element of the GRAS determination.

Thus, it is concluded that these uses of D-psicose (D-allulose) is GRAS based on scientific procedures and that other experts qualified to assess the safety of foods and food ingredients would concur with these conclusions. Therefore, not only is the proposed use of D-allulose safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also Generally Recognized as Safe (GRAS) according to Title 21 Code of Federal Regulations (21 CFR).

6.F. Discussion of Information Inconsistent with GRAS Determination

We are not aware of information that would be considered inconsistent with the finding that the proposed use of D-psicose (D-allulose) preparations in foods and beverages meeting appropriate specifications and cGMP is GRAS.

Daesang: D-psicose (synonymous with D-allulose)

PART 7. DATA AND INFORMATION ARE GENERALLY AVAILABLE

7.A. Data and Information are Generally Available

Altug T. 2003. Introduction to Toxicology and Food. Boca Raton, FL: CRC Press.

An M, Lee J, Park YC, Park C, Kim HJ. 90-Day repeated oral toxicity test of D-psicose produced from *Microbacterium foliorum*. Regul Toxicol Pharmacol. 2019;109:104485.

Atiee GJ, Hinit NK, Zamora CA, Michael R. Natalino,MR, Smeberg, V, Juren J, Ilinitt S, Mutter W, Benedikt RA. 2015. An open-label, single-dose, nonrandomized microtracer study to determine the mass balance of orally administered, ¹⁴C-labeled sweetener product with 15 g sweetener in healthy adult subjects. In Tate & Lyle Citizens' petition to US FDA (Attachment A; 3007423-synopsis-pages 1 - 6). Available at: <https://www.regulations.gov/document/FDA-2019-D-0725-0005>.

A summary of this study was published in Williamson et al. (2014) as an abstract. Williamson P, Schunk T, Woodyer R, Chiu D, Song Q, Atiee G, Unger S. A single-dose, microtracer study to determine the mass balance of orally administered, ¹⁴C-labeled sweetener in healthy adult men (LB450). FASEB J. 2014; 28(51). Available at: https://doi.org/10.1096/fasebj.28.1_supplement.lb450.

Baek SH, Park SJ, Lee HG. D-psicose, a sweet monosaccharide, ameliorates hyperglycemia and dyslipidemia in C57BL/6J db/db mice. J Food Sci. 2010;75:H49-53.

Bär A. Characteristics and significance of D-tagatose-induced liver enlargement in rats: An interpretative review. Regul Toxicol Pharmacol. 1999; 29:S83-S93.

Braunstein CR, Noronha JC, Khan TA, Mejia SB, Wolever TM, Josse RG, Kendall CW, Sievenpiper JL. Effect of fructose and its epimers on postprandial carbohydrate metabolism: A systematic review and meta-analysis. Clin Nutr. 2020;39:3308-3318.

Chen J, Huang W, Zhang T, Lu M, Jiang B. Anti-obesity potential of rare sugar D-psicose by regulating lipid metabolism in rats. Food Funct. 2019;10:2417-25.

Chung YM, Hyun Lee J, Youl Kim D, Hwang SH, Hong YH, Kim SB, Jin Lee S, Hye Park C. Dietary D-psicose reduced visceral fat mass in high-fat diet-induced obese rats. J Food Sci. 2012;77:H53-58.

European Food Safety Authority (EFSA). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 13: suitability of taxonomic units notified to EFSA until September 2020. EFSA J. 2021;19(1):6377.

Food and Drug Administration (FDA). 2012. GRN 400. D-psicose, filed by CJ CheilJedang, South Korea. Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=400>.

Daesang: D-psicose (synonymous with D-allulose)

FDA. 2014. GRN 498. D-psicose, filed by Matsutani Chemical Industry, Japan. Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=498>

FDA. 2017. GRN 693. D- psicose, filed by Samyang Corp., South Korea. Available at: GRAS Notice 693, D-psicose (fda.gov).

FDA. 2020. GRN 828, D-psicose, filed by Samyang Corp., South Korea. Available at: GRAS Notice 828, D-psicose (fda.gov).

FDA, 2021. GRN 932. 2-fusocyllactose, filed by A Advanced Protein Technologies Corp., S. Korea. Available at. GRAS Notice 932 Agency Response Letter (fda.gov).

FDA. 2023. GRN 1024. D-psicose, filed by Blue California, USA. Available at: GRAS Notice 1024, D-psicose (FDA.gov).

Gou Y, Liu B, Cheng M, Yamada T, Iida T, Wang S, Banno R, Koike T. D-allulose ameliorates skeletal muscle insulin resistance in high-fat diet-fed rats. *Molecules*. 2021;26:6310.

Han Y, Choi BR, Kim SY, Kim SB, Kim YH, Kwon EY, Choi MS. Gastrointestinal tolerance of D-allulose in healthy and young adults. A non-randomized controlled trial. *Nutrients*. 2018a;10:2010.

Han Y, Kwon EY, Yu MK, Lee SJ, Kim HJ, Kim SB, Kim YH, Choi MS. A preliminary study for evaluating the dose-dependent effect of D-allulose for fat mass reduction in adult humans: a randomized, double-blind, placebo-controlled trial. *Nutrients*. 2018b;10. pii: E160.

Han Y, Park H, Choi BR, Ji Y, Kwon EY, Choi MS. Alteration of microbiome profile by d-allulose in amelioration of high-fat-diet-induced obesity in mice. *Nutrients*. 2020;12:352.

Hayashi N, Iida T, Yamada T, Okuma K, Takehara I, Yamamoto T, Yamada K, Tokuda M. Study on the postprandial blood glucose suppression effect of D-psicose in borderline diabetes and the safety of long-term ingestion by normal human subjects. *Biosci Biotechnol Biochem*. 2010;74:510-19.

Hobbs L. Sweeteners from starch production, properties and uses *In: Starch Chemistry and Technology*, Third Edition, Elsevier, 2009: pp 797–832.

Hossain A, Yamaguchi F, Hirose K, Matsunaga T, Sui L, Hirata Y, Noguchi C, Katagi A, Kamitori K, Dong Y, Tsukamoto I, Tokuda M. Rare sugar D-psicose prevents progression and development of diabetes in T2DM model Otsuka Long-Evans Tokushima fatty rats. *Drug Des Devel Ther*. 2015a;9:525-35.

Hossain A, Yamaguchi F, Matsuo T, Tsukamoto I, Toyoda Y, Ogawa M, Nagata Y, Tokuda M. Rare sugar D-psicose: Potential role and therapeutic monitoring in maintaining obesity and type 2 diabetes mellitus. *Pharmacol Ther*. 2015b;155:49-59.

Daesang: D-psicose (synonymous with D-allulose)

- Hossain A, Yamaguchi F, Matsunaga T, Hirata Y, Kamitori K, Dong Y, Sui L, Tsukamoto I, Ueno M, Tokuda M. Rare sugar D-psicose protects pancreas β -islets and thus improves insulin resistance in OLETF rats. *Biochem Biophys Res Commun.* 2012;425:717-23.
- Huntington Lab, 2011, cited in GRN 400, US FDA, 2011. Original reports or details of the studies are not available.
- Iida T, Hayashi N, Yamada T, Yoshikawa Y, Miyazato S, Kishimoto Y, Okuma K, Tokuda M, Izumori K. Failure of D-psicose absorbed in the small intestine to metabolize into energy and its low large intestinal fermentability in humans. *Metabolism.* 2010;59:206-14.
- Iida T, Kishimoto Y, Yoshikawa Y, Okuma K, Yagi K, Matsuo T, Izumori K. Estimation of maximum non-effective level of D-allulose in causing diarrhea in human subjects. *J Adv Food Inged.* 2007;10:15-19 (written in Japanese with English abstract; described in GRN 400).
- Itoh K, Mizuno S, Hama S, Oshima W, Kawamata M, Hossain A, Ishihara Y, Tokuda M. Beneficial effects of supplementation of the rare sugar "D-allulose" against hepatic steatosis and severe obesity in *Lep^{ob}/Lep^{ob}* mice. *J Food Sci.* 2015;80:H1619-26.
- Kanasaki A, Niibo M, Iida T. Effect of D-allulose feeding on the hepatic metabolomics profile in male Wistar rats. *Food Funct.* 2021;12:3931-38.
- Kanasaki K, Jiang Z, Mizokami T, Shirouchi T, Iida T, Nagata Y, Sato M. Dietary D- allulose alters cholesterol metabolism in Golden Syrian hamsters partly by reducing serum PCSK9 levels. *J Funct Foods.* 2019; 60:103429.
- Kim SE, Kim SJ, Kim HJ, Sung MK. D-psicose, a sugar substitute, suppresses body fat deposition by altering networks of inflammatory response and lipid metabolism in C57BL/6J-ob/ob mice. *J Func Foods.* 2017;28: 265-74.
- Kim H, Park C, Sa S, Case I, Li C, Gao Y, Wang H, Tian J. A study of D-allulose-associated reproductive toxicity in rats. *Food Chem Toxicol.* 2019;131:110548.
- Kishida K, Martinez G, Ida T, Yamada T, Ferraris RP, Toyoda Y. D-allulose is a substrate of glucose transporter type 5 (GLUT5) in the small intestine. *Food Chem.* 2019; 277: 604-8.
- Lambe J, Kearney J. The influence of survey duration on estimates of food intakes -Relevance for food-based dietary guidelines: *Br J Nutr.* 1999;81 Suppl 2:S139-42.
- Lee D, Han Y, Kwon EY, Choi MS. D-allulose ameliorates metabolic dysfunction in C57BL/KsJ-db/db mice. *Molecules* 2020; 25: 3656.
- Lee GH, Peng C, Lee HY, Park SA, Hoang TH, Kim JH, Sa S, Kim GE, Han JS, Chae HJ. D-allulose ameliorates adiposity through the AMPK-SIRT1-PGC-1 α pathway in HFD-induced SD rats. *Food Nutr Res.* 2021;65.

Daesang: D-psicose (synonymous with D-allulose)

- Matsuo T, Baba Y, Hashiguchi M, Takeshita K, Izumori K, Suzuki H. Less body fat accumulation with D-psicose diet versus D-fructose diet. *J Clin Biochem Nutr.* 2001a;30:55-65.
- Matsuo T, Baba Y, Hashiguchi M, Takeshita K, Izumori K, Suzuki H. Dietary D-psicose, a C-3 epimer of D-fructose, suppresses the activity of hepatic lipogenic enzymes in rats. *Asia Pac J Clin Nutr.* 2001b;10:233-37.
- Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Effects of oral acute administration and subchronic feeding of several levels of D-psicose in rats. *J Nutr Sci Vitaminol.* 2002;48:512-16.
- Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Metabolic effects of D-psicose in rats: Studies on fecal and urinary excretion and cecal fermentation. *Asia Pac J Clin Nutr.* 2003;12:225-31.
- Matsuo T, Izumori K. Effects of supplemental D-psicose on glucose tolerance and serum adipocytokine levels in rats fed a high-fat diet or a low-fat diet. *J. Oleo Sci.* 2004;53:453-60.
- Matsuo T, Izumori K. Effects of dietary D-psicose on diurnal variation in plasma glucose and insulin concentrations of rats. *Biosci Biotechnol Biochem.* 2006;70:2081-85.
- Matsuo T, Ishii R, Shirai Y. The 90-day oral toxicity of D-psicose in male Wistar rats. *J Clin Biochem Nutr.* 2012;50:158-61.
- Nagata Y, Kanasaki A, Tamaru S, Tanaka K. D-Psicose, an epimer of D-fructose, favorably alters lipid metabolism in Sprague-Dawley rats. *J Agric Food Chem.* 2015;63:3168-76.
- Nishii N, Takashima S, Kobatake Y, Tokuda M, Kitagawa H. The long-term safety of D-allulose administration in healthy dogs. *J Vet Med Sci.* 2017; 79:1780-84.
- Ochiai M, Nakanishi Y, Yamada T, Iida T, Matsuo T. Inhibition by dietary D-psicose of body fat accumulation in adult rats fed a high-sucrose diet. *Biosci Biotechnol Biochem.* 2013;77:1123-26.
- Ochiai M, Onishi K, Yamada T, Iida T, Matsuo T. D-Psicose increases energy expenditure and decreases body fat accumulation in rats fed a high-sucrose diet. *Int J Food Sci Nutr.* 2014; 65:245-50.
- Oshima H, Kimura I, Izumori K. Psicose contents in various food products and its origin. *Food Sci Technol Res.* 2006;12:137-43.
- Pongkan W, Jinawong K, Pratchayasakul W, Jaiwongkam T, Kerdphoo S, Tokuda M, Chattipakorn SC, Chattipakorn N. D- allulose provides cardioprotective effect by attenuating cardiac mitochondrial dysfunction in obesity-induced insulin-resistant rats. *Eur J Nutr.* 2021;60:2047-61.

Daesang: D-psicose (synonymous with D-allulose)

Prachayasakul W, Jinawong K, Pongkan W, Jaiwongkam T, Arunsak B, Chunchai T, Tokuda M, Chattipakorn N, Chattipakorn SC. Not only metformin, but also D- allulose, alleviates metabolic disturbance and cognitive decline in prediabetic rats. *Nutr Neurosci*. 2022;25:1115-27.

Sa S, Seol Y, Lee AW, Heo Y, Kim HJ, Park CJ. Teratogenicity of D-allulose. *Toxicol Rep*. 2022;9:821-24.

Tanaka M, Kanasaki A, Hayashi N, Iida T, Murao K. Safety and efficacy of a 48-week long-term ingestion of D-allulose in subjects with high LDL cholesterol levels. *Fundamental Toxicol Sci*. 2020;7:1-15.

Tanaka M, Kanasaki A, Hayashi N, Iida T, Murao K. The influence of long-term ingestion of D-allulose in hypercholesterolemia patients under statin therapy. *Fundamental Toxicol Sci*. 2021;8:23-31.

Tsukamoto I, Hossain A, Yamaguchi F, Hirata Y, Dong Y, Kamitori K, Sui L, Nonaka M, Ueno M, Nishimoto K, Suda H, Morimoto K, Shimonishi T, Saito M, Song T, Konishi R, Tokuda M. Intestinal absorption, organ distribution, and urinary excretion of the rare sugar D-psicose. *Drug Design Devel Ther*. 2014;8:1955-64.

Whistler RL, Singh PP, Lake WC. D-psicose metabolism in the rat. *Carbohydr Res*. 1974; 34: 200-02.

Yagi K, Matsuo T. The study on long-term toxicity of D-psicose in rats. *J Clin Biochem Nutr*. 2009;45:271-77.

7.B. Data and Information Are Not Generally Available

Appendix E. Toxicological Evaluation of Daesang's D-psicose (D-allulose)

This report summarizes the following studies:

Bacterial reverse mutation study, Biototech, 2022a

In vitro mammalian chromosomal aberration test, Biototech, 2022b

In vivo mouse micronucleus test, Biototech, 2022c

Acute toxicity in rats, Biototech, 2022d.

The results of these studies confirm previous findings and do not deserve publication as new discoveries.

Daesang: D-psicose (synonymous with D-allulose)

APPENDIX A. CERTIFICATE OF ANALYSIS



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CHEONBUK KOREA
Phone : 82-63-472-6130
Fax No : 82-63-471-8807

CERTIFICATE OF ANALYSIS

Product : Allulose syrup
Lot No : DSAS220814
Quantity :
Mfd. Date : 2022-08-14
Expiry Date : 1 years from Manufacturing date

DATE : 2022-09-28

TEST ITEM	SPECIFICATION	RESULT	TEST METHOD
Appearance	Light yellow liquid	Clear liquid	Visual
Odor	Odorless	Odorless	-
Allulose content (% D.S)	≥ 95.0	97.9	HPLC
Brix (% w/wt)	≥ 63.0	65.3	Brix meter
pH (10% Soln)	3.0 - 7.0	6.6	pH meter
Ash (% w/wt)	≤ 0.5	< 0.01	AOAC 900.02C
Pb ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
As ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Cd ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Total plate count (CFU/g)	≤ 1,000	< 10	AOAC 990.12
Coliforms (CFU/10g)	< 10	< 10	AOAC 991.14
Yeast and Mold (CFU/g)	≤ 100	< 10	AOAC 997.02
Salmonella (CFU/25g)	Negative	Negative	AOAC 999.09
Staphylococcus aureus (CFU/g)	< 10	< 10	AOAC 2001.05

QA TEAM LEADER

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Daesang: D-psicose (synonymous with D-allulose)



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 Fax No : 82-63-471-8807

CERTIFICATE OF ANALYSIS

Product : **Allulose syrup**
 Lot No : **DSAS220819**
 Quantity :
 Mfd. Date : 2022-08-19
 Expiry Date : 1 years from Manufacturing date

DATE : 2022-09-28

TEST ITEM	SPECIFICATION	RESULT	TEST METHOD
Appearance	Light yellow liquid	Clear liquid	Visual
Odor	Odorless	Odorless	-
Allulose content (%) (D.S)	≥ 95.0	98.1	HPLC
Brix (% w/w)	≥ 63.0	68.6	Brix meter
pH (10% Soln)	3.0 - 7.0	6.6	pH meter
Ash (% w/w)	≤ 0.5	< 0.01	AOAC 900.02C
Pb ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
As ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Cd ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Total plate count (CFU/g)	≤ 1,000	< 10	AOAC 990.12
Coliforms (CFU/10g)	< 10	< 10	AOAC 991.14
Yeast and Mold (CFU/g)	≤ 100	< 10	AOAC 997.02
Salmonella (CFU/25g)	Negative	Negative	AOAC 999.09
Staphylococcus aureus (CFU/g)	< 10	< 10	AOAC 2001.05



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Daesang: D-psicose (synonymous with D-allulose)



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CERTIFICATE OF ANALYSIS

Product : Allulose syrup
Lot No : DSAS220823
Quantity :
Mfd. Date : 2022-08-23
Expiry Date : 1 years from Manufacturing date

DATE : 2022-09-28

TEST ITEM	SPECIFICATION	RESULT	TEST METHOD
Appearance	Light yellow liquid	Clear liquid	Visual
Odor	Odorless	Odorless	-
Allulose content (%) (D.S)	≥ 95.0	97.9	HPLC
Brix (%) (wt/wt)	≥ 63.0	66.6	Brix meter
pH (10% Soln)	3.0 - 7.0	6.4	pH meter
Ash (%) (wt/wt)	≤ 0.5	< 0.01	AOAC 900.02C
Pb ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
As ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Cd ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Total plate count (CFU/g)	≤ 1,000	< 10	AOAC 990.12
Coliforms (CFU/10g)	< 10	< 10	AOAC 991.14
Yeast and Mold (CFU/g)	≤ 100	< 10	AOAC 997.02
Salmonella (CFU/25g)	Negative	Negative	AOAC 999.09
Staphylococcus aureus (CFU/g)	< 10	< 10	AOAC 2001.05

QA TEAM LEADER

DAESANG CORPORATION

Daesang: D-psicose (synonymous with D-allulose)



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 Fax No : 82-63-471-8807

CERTIFICATE OF ANALYSIS

Product : Allulose crystal
 Lot No : DSAC220819
 Quantity :
 Mfd. Date : 202208-19
 Expiry Date : 1 years from Manufacturing date

DATE : 2022-09-28

TEST ITEM		SPECIFICATION	RESULT	TEST METHOD
Appearance		White powder	White powder	Visual
Odor		Odorless	Odorless	-
Allulose content	(%, D.S)	≥ 98.0	99.6	HPLC
Moisture	(%)	≤ 5.0	0.62	AOAC 977.21
pH (10% Soln)		3.0 - 7.0	6.6	pH meter
Ash	(%, wt/wt)	≤ 0.5	< 0.01	AOAC 900.02C
Pb	ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
As	ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Cd	ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Total plate count	(CFU/g)	≤ 1,000	< 10	AOAC 990.12
Coliforms	(CFU/10g)	< 10	< 10	AOAC 991.14
Yeast and Mold	(CFU/g)	≤ 100	< 10	AOAC 997.02
Salmonella	(CFU/25g)	Negative	Negative	AOAC 999.09
Staphylococcus aureus	(CFU/g)	< 10	< 10	AOAC 2001.05



QA TEAM LEADER

DAESANG CORPORATION



DAESANG CORPORATION

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STARCH & SWEETNER BUSINESS UNIT
 GUNSAN PLANT
 884, OEHANG-RO, GUNSAN,
 CHEONBUK, KOREA
 Phone 0 82-63-472-6130
 Fax No : 82-63-471-8807

CERTIFICATE OF ANALYSIS

Product : Allulose crystal
 Lot No : DSAC220823
 Quantity :
 Mfd. Date : 2022-08-23
 Expiry Date : 1 years from Manufacturing date

DATE : 2022-09-28

TEST ITEM		SPECIFICATION	RESULT	TEST METHOD
Appearance		White powder	White powder	Visual
Odor		Odorless	Odorless	-
Allulose content	(%, D.S)	≥ 98.0	99.8	HPLC
Moisture	(%)	≤ 5.0	0.62	AOAC 977.21
pH (10% Soln)		3.0 - 7.0	6.4	pH meter
Ash	(%, wt/wt)	≤ 0.5	< 0.01	AOAC 900.02
Pb	ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
As	ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Cd	ppm	≤ 0.5	< 3.5 ppb	AOAC 2015.01
Total plate count	(CFU/g)	≤ 1,000	< 10	AOAC 990.12
Coliforms	(CFU/10g)	< 10	< 10	AOAC 991.14
Yeast and Mold	(CFU/g)	≤ 100	< 10	AOAC 997.02
Salmonella	(CFU/25g)	Negative	Negative	AOAC 999.09
Staphylococcus aureus	(CFU/g)	< 10	< 10	AOAC 2001.05



QA TEAM LEADER

DAESANG CORPORATION



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Order # Sample ID: 2022-009808-04 **Company:** Daesang Corporation
Customer Sample ID: Allulose syrup -1
Sample Description: Lot No.: DSAS220814

Analytical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² Allulose	Allulose	65.4%	25-Oct-2022
³ Ash	Ash	<0.012 %	11-Oct-2022
³ Metals (DMA)	Mercury	<2.00 ppb	06-Oct-2022
³ Metals (ICP-MS)	Arsenic	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Cadmium	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Lead	<10 ppb	10-Oct-2022
³ Moisture by Vacuum Oven	Moisture	31.595 %	13-Oct-2022
² pH	pH	5.44	12-Oct-2022
	Dilution	2 %	12-Oct-2022

Micro Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
³ Aerobic Plate Count using PCA	Aerobic Plate Count	<10 CFU / g	07-Oct-2022
³ Coliform using Petrifilm	Coliform count	<10 CFU / g	06-Oct-2022
³ E. coli using Petrifilm	E. coli	<10 CFU / g	07-Oct-2022
³ Mold using DRBC	Mold	<10 CFU / g	10-Oct-2022
³ Salmonella	Salmonella	Negative / 25 grams	11-Oct-2022
³ Staphylococcus aureus using Petrifilm	Staphylococcus aureus	<10 CFU / g	06-Oct-2022
³ Yeast using DRBC	Yeast	<10 CFU / g	10-Oct-2022

Physical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² BRIX	BRIX	65.4 °	07-Oct-2022

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³ Reported value has been revised from original report # 64987



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Order # Sample ID: 2022-009808-05 **Company:** Daesang Corporation
Customer Sample ID: Allulose syrup -2
Sample Description: Lot No.: DSAS220819

Analytical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² Allulose	Allulose	68.6%	25-Oct-2022
³ Ash	Ash	<0.012 %	11-Oct-2022
³ Metals (DMA)	Mercury	<2.00 ppb	06-Oct-2022
³ Metals (ICP-MS)	Arsenic	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Cadmium	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Lead	<10 ppb	10-Oct-2022
³ Moisture by Vacuum Oven	Moisture	28.104 %	13-Oct-2022
² pH	pH	5.49	12-Oct-2022
	Dilution	2 %	12-Oct-2022

Micro Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
³ Aerobic Plate Count using PCA	Aerobic Plate Count	<10 CFU / g	07-Oct-2022
³ Coliform using Petrifilm	Coliform count	<10 CFU / g	06-Oct-2022
³ E. coli using Petrifilm	E. coli	<10 CFU / g	07-Oct-2022
³ Mold using DRBC	Mold	<10 CFU / g	10-Oct-2022
³ Salmonella	Salmonella	Negative / 25 grams	11-Oct-2022
³ Staphylococcus aureus using Petrifilm	Staphylococcus aureus	<10 CFU / g	06-Oct-2022
³ Yeast using DRBC	Yeast	<10 CFU / g	10-Oct-2022

Physical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² BRIX	BRIX	68.6 °	07-Oct-2022

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³ Reported value has been revised from original report # 64987



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Order # Sample ID: 2022-009808-06 **Company:** Daesang Corporation
Customer Sample ID: Allulose syrup -3
Sample Description: Lot No.: DSAS220823

Analytical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² Allulose	Allulose	65.4%	25-Oct-2022
³ Ash	Ash	<0.012 %	11-Oct-2022
³ Metals (DMA)	Mercury	<2.00 ppb	06-Oct-2022
³ Metals (ICP-MS)	Arsenic	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Cadmium	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Lead	<10 ppb	10-Oct-2022
³ Moisture by Vacuum Oven	Moisture	30.848 %	13-Oct-2022
² pH	pH	5.42	12-Oct-2022
	Dilution	2 %	12-Oct-2022

Micro Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
³ Aerobic Plate Count using PCA	Aerobic Plate Count	100 est. CFU / g	07-Oct-2022
³ Coliform using Petrifilm	Coliform count	<10 CFU / g	06-Oct-2022
³ E. coli using Petrifilm	E. coli	<10 CFU / g	07-Oct-2022
³ Mold using DRBC	Mold	<10 CFU / g	10-Oct-2022
³ Salmonella	Salmonella	Negative / 25 grams	11-Oct-2022
³ Staphylococcus aureus using Petrifilm	Staphylococcus aureus	<10 CFU / g	06-Oct-2022
³ Yeast using DRBC	Yeast	<10 CFU / g	10-Oct-2022

Physical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² BRIX	BRIX	66.1 °	07-Oct-2022

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³ Reported value has been revised from original report # 64987

Daesang: D-psicose (synonymous with D-allulose)



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Order # Sample ID: 2022-009808-01 **Company:** Daesang Corporation
Customer Sample ID: Allulose crystal -1
Sample Description: Lot No.: DSAC220819

Analytical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² Allulose	Allulose	99.7%	25-Oct-2022
Ash	Ash	<0.012 %	11-Oct-2022
Metals (DMA)	Mercury	<4.00 ppb	06-Oct-2022
Metals (ICP-MS)	Arsenic	<10 ppb	10-Oct-2022
Metals (ICP-MS)	Cadmium	<10 ppb	10-Oct-2022
Metals (ICP-MS)	Lead	<10 ppb	10-Oct-2022
Moisture by Vacuum Oven	Moisture	<0.012 %	12-Oct-2022
² pH	pH	6.26	12-Oct-2022
	Dilution	2 %	12-Oct-2022

Micro Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
Aerobic Plate Count using PCA	Aerobic Plate Count	40 est. CFU / g	07-Oct-2022
Coliform using Petrifilm	Coliform count	<10 CFU / g	06-Oct-2022
E. coli using Petrifilm	E. coli	<10 CFU / g	07-Oct-2022
Mold using DRBC	Mold	10 est. CFU / g	10-Oct-2022
Salmonella	Salmonella	Negative / 25 grams	11-Oct-2022
Staphylococcus aureus using Petrifilm	Staphylococcus aureus	<10 CFU / g	06-Oct-2022
Yeast using DRBC	Yeast	<10 CFU / g	10-Oct-2022

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Order # Sample ID: 2022-009808-02 **Company:** Daesang Corporation
Customer Sample ID: Allulose crystal -2
Sample Description: Lot No.: DSAC220823

Analytical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² Allulose	Allulose	99.2%	25-Oct-2022
³ Ash	Ash	<0.012 %	11-Oct-2022
³ Metals (DMA)	Mercury	<4.00 ppb	06-Oct-2022
³ Metals (ICP-MS)	Arsenic	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Cadmium	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Lead	<10 ppb	10-Oct-2022
³ Moisture by Vacuum Oven	Moisture	<0.012 %	12-Oct-2022
² pH	pH	5.71	12-Oct-2022
	Dilution	2 %	12-Oct-2022

Micro Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
³ Aerobic Plate Count using PCA	Aerobic Plate Count	<10 CFU / g	07-Oct-2022
³ Coliform using Petrifilm	Coliform count	<10 CFU / g	06-Oct-2022
³ E. coli using Petrifilm	E. coli	<10 CFU / g	07-Oct-2022
³ Mold using DRBC	Mold	<10 CFU / g	10-Oct-2022
³ Salmonella	Salmonella	Negative / 25 grams	11-Oct-2022
³ Staphylococcus aureus using Petrifilm	Staphylococcus aureus	<10 CFU / g	06-Oct-2022
³ Yeast using DRBC	Yeast	<10 CFU / g	10-Oct-2022

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³ Reported value has been revised from original report # 64987



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Order # Sample ID: 2022-009808-03 **Company:** Daesang Corporation
Customer Sample ID: Allulose crystal -3
Sample Description: Lot No.: DSAC220906

Analytical Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
² Allulose	Allulose	100.3%	25-Oct-2022
³ Ash	Ash	<0.012 %	11-Oct-2022
³ Metals (DMA)	Mercury	<4.00 ppb	06-Oct-2022
³ Metals (ICP-MS)	Arsenic	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Cadmium	<10 ppb	10-Oct-2022
³ Metals (ICP-MS)	Lead	<10 ppb	10-Oct-2022
³ Moisture by Vacuum Oven	Moisture	<0.012 %	12-Oct-2022
² pH	pH	5.73	12-Oct-2022
	Dilution	2 %	12-Oct-2022

Micro Testing

<u>Method:</u>	<u>Component:</u>	<u>Result:</u>	<u>Test Date:</u>
³ Aerobic Plate Count using PCA	Aerobic Plate Count	<10 CFU / g	07-Oct-2022
³ Coliform using Petrifilm	Coliform count	<10 CFU / g	06-Oct-2022
³ E. coli using Petrifilm	E. coli	<10 CFU / g	07-Oct-2022
³ Mold using DRBC	Mold	<10 CFU / g	10-Oct-2022
³ Salmonella	Salmonella	Negative / 25 grams	11-Oct-2022
³ Staphylococcus aureus using Petrifilm	Staphylococcus aureus	<10 CFU / g	06-Oct-2022
³ Yeast using DRBC	Yeast	<10 CFU / g	10-Oct-2022

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³ Reported value has been revised from original report # 64987

Daesang: D-psicose (synonymous with D-allulose)



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Analytical Method References:

Method Name

Method Reference

Allulose	Please contact for Method Details
Ash	AOAC: 923.03*
Metals (DMA)	EPA 7473
Metals (ICP-MS)	AOAC: 993.14*, AOAC: 2015.06*
Moisture by Vacuum Oven	AOAC: 945.43*, 934.01*
pH	AACC 02-52*, AOAC 943.02* and 981.12*

Micro Method References:

Method Name

Method Reference

Aerobic Plate Count using PCA	FDA BAM CH 3*
Coliform using Petrifilm	AOAC 991.14
E. coli using Petrifilm	AOAC 991.14
Mold using DRBC	FDA BAM CH 18*, Compendium*
Salmonella	AOAC 2013.01*
Staphylococcus aureus using Petrifilm	AOAC 2003.07
Yeast using DRBC	FDA BAM CH 18*, Compendium*

Physical Testing Method

Method Name

Method Reference

BRIX	Please Contact for Method Details
------	-----------------------------------

* This method has been modified.

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Report #: 64987.6

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Daesang: D-psicose (synonymous with D-allulose)

APPENDIX B. Absence of production strain from final product



1/15
REPORT T1493R1556/2022
24.1.2023

1

STUDY REPORT

2

Analysis of the absence of *Corynebacterium glutamicum* KCCM 80101

3

production strain from allulose crystal fermentation product

4

Author

Approved by

5

6

Biosafe – Biological Safety Solutions Ltd

7

Microkatu 1M

8

FIN-70210 KUOPIO, Finland

9

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10

Requested by

11

Daesang Corp.

12

Jongno-gu, Changgyeonggung-ro

13

120 Seoul

14

Republic of Korea

15

This report contains scientific interpretation of the received data by the named scientists in the

16

Biosafe Ltd. It does not necessarily represent the official views of the competent authorities.

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29	background contamination in the test material.....	7
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32	Appendix 1. Method to detect viable cells of the production strain in the test material product batches	
33	(Confidential).....	12
34		

35 NOTE
36 The samples will be stored for three months after reporting the results.
37 Copies of all the laboratory data are available to the client on request.

38 EXECUTIVE SUMMARY

39 *Scope*

40 Daesang Corp. requested Biosafe Ltd to analyse the absence of the production strain *Corynebacterium*
41 *glutamicum* KCCM 80101 (hereafter production strain) in the allulose crystal fermentation product
42 (hereafter test material) in compliance with the EFSA FEEDAP Panel (2018) Guidance on the
43 characterisation of microorganisms used as feed additives or as production organisms, Section 3.1.
44 Absence of the production strain.

45 *Strategy*

46 The absence of the production strain was analysed using a culture-based method. The need for and
47 type of selective pressure against possible contaminating microbiota in the samples were tested.
48 Culturing time of the production strain under the selected conditions in the absence of the product was
49 determined. The absence of viable cells of the production strain was analysed from a total of 9 grams
50 of three independent production batches, each sampled in triplicate. Controls with samples from each
51 of the three test material batches spiked with low counts of viable production strain cells were included
52 to confirm that the test material did not inhibit the growth of the production strain, if present in the
53 samples. Extended culturing time was applied to enable recovery of possible stressed cells in the
54 samples.

55 *Results*

56 The culturing time of the production strain at 30 °C was two days on selective agar. Test material
57 samples spiked with production strain verified that the medium and culturing conditions enabled the
58 growth of possible production strain cells remaining in the product, and that the product itself did not
59 inhibit the growth of the production strain cells. No colonies were detected from 9 grams of the product.

60 *Conclusion*

61 The absence of viable cells of the production strain was demonstrated in nine samples, three from each
62 of the three batches. Each of the nine samples of 1 g was taken from 10-g subsamples. Altogether, 9 g
63 of test material were analysed. No production strain colonies were detected.

64 **1 INTRODUCTION**

65 Daesang Corp. requested Biosafe Ltd to analyse the absence of the production strain *Corynebacterium*
66 *glutamicum* KCCM 80101 (hereafter production strain) in the allulose crystal fermentation product
67 (hereafter test material) in compliance with the EFSA Guidance on the characterisation of
68 microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018).

69 **2 COMPLIANCE WITH THE EFSA GUIDANCE**

70 The method development and verification followed the EFSA Guidance on the characterisation of
71 microorganisms used as feed additives or as production organisms, Section 3.1 (Absence of the
72 production strain) (EFSA FEEDAP Panel, 2018). According to the Guidance, the absence of the production
73 strain should be investigated by means of a culture-based method targeted to the detection of viable
74 cells.

75 The procedure should enable the recovery of stressed cells by cultivation in or onto media with minimal
76 selective pressure and/or by providing a longer (at least two times) incubation time compared to the
77 normal culturing time.

78 The detection should consider selectivity against contaminating microbiota possibly occurring in the
79 samples in case the contamination interferes with the detection of the production strain.

80 A positive control with samples spiked with low counts of viable cells of the production strain should
81 be included to prove that the medium and cultivation conditions enable growth of any possible viable
82 cells remaining in the product.

83 The absence of viable cells of the production strain should be analysed from at least nine samples
84 obtained from a minimum of three independent batches of the final product. The absence of viable cells
85 of the production strain should be demonstrated in a volume corresponding to at least 1 g or mL of
86 product, obtained from a sample of at least 10 g or mL.

87 **3 TEST MATERIAL, DATA AND METHODS**

88 **3.1 Test material**

89 The test material and the viable production strain were provided to Biosafe Ltd by Daesang Corp. Three
90 test material batches and the test material production strain were provided (Appendix 1, Table A1). The
91 results apply to the test material as received. Essential dates related to notification of the study
92 according to transparency regulation (EU 2019/1381) are described in Appendix 1, Table A1.

93 **3.2 Test design**

94 Summary of the test design is shown in Table 1. Three independent batches of test material were
95 analysed in triplicate. Each of the nine samples of 1 g was taken from 10-g subsamples. Altogether, 9 g
96 of test material were analysed

97

98

99

100 **Table 1.** Summary of the test design

Purpose of test	Experiment	Test material	Production strain
Demonstrating the absence of the production strain using extended culturing time	Analysis of test material	Three batches in triplicate	No
Demonstrating the viability of the production strain in the presence of test material	Spiked controls	1 sample from each batch	Yes
Demonstrating the viability of the production strain under the test conditions	Positive control	No	Yes
Demonstrating the sterility of the used diluent and experimental process	Negative control	No	No

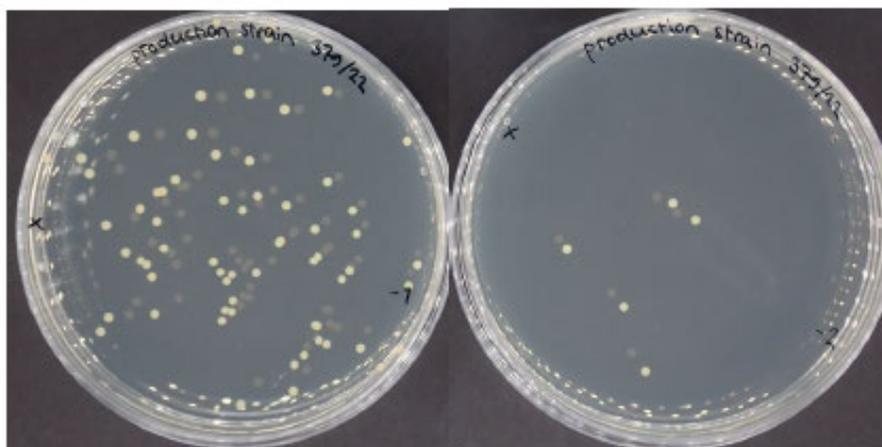
101 **3.3 Methods**

102 The test was performed according to the EFSA FEEDAP Panel (2018) Guidance using a culture-based
103 method for the detection of viable cells. The procedure enables the recovery of stressed cells and
104 considers specificity against possible contaminating microbiota in the sample. The methods are
105 described in detail in Appendix 1 (confidential).

106 **4 RESULTS AND DISCUSSION**

107 **4.1 Normal culturing time of the production strain**

108 The normal culturing time of the production strain on agar used in the analysis at 30 °C was two days
109 (Figure 1). The colony count of the suspension used for spiking was 5.8×10^2 CFU/mL.

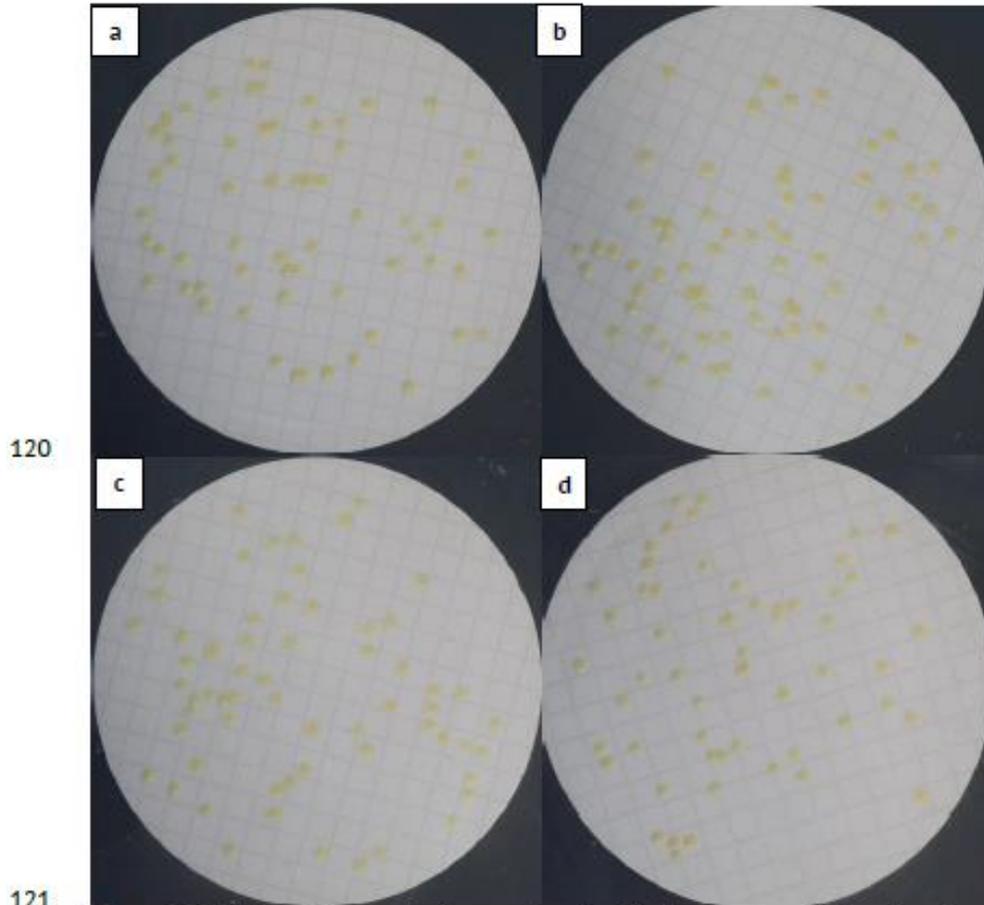


110 **Figure 1.** Incubation of the production strain for two days on agar used in the analysis at 30 °C. A 100-
111 µL aliquot of nondiluted (left) and 1:10 diluted (right) cell suspension was spread on each dish.
112

113 **4.2 Spiked controls: Viability of the production strain in the presence of test material**

114 The viability of the production strain in the presence of the test material was analysed (Figures 2 and
115 3). The control experiment with spiked samples was performed with target cell count of production
116 strain of approximately 58 CFU/filter. Spiked samples showed an average of 64 CFU/filter corresponding
117 to 1 g of test material (Fig. 3 a-c), proving that the medium and cultivation conditions enabled growth
118 of viable cells remaining in the product and that the test material did not inhibit the growth of the
119 production strain. Selective pressure was used.

Daesang: D-psicose (synonymous with D-allulose)



121
122 Figure 2. Viability of the production strain spiked in the test material from batches 1 (filter a), 2 (filter
123 b) and 3 (filter c). Filter d represents positive control (production strain but no test material). Incubation
124 at 30 °C, two days.

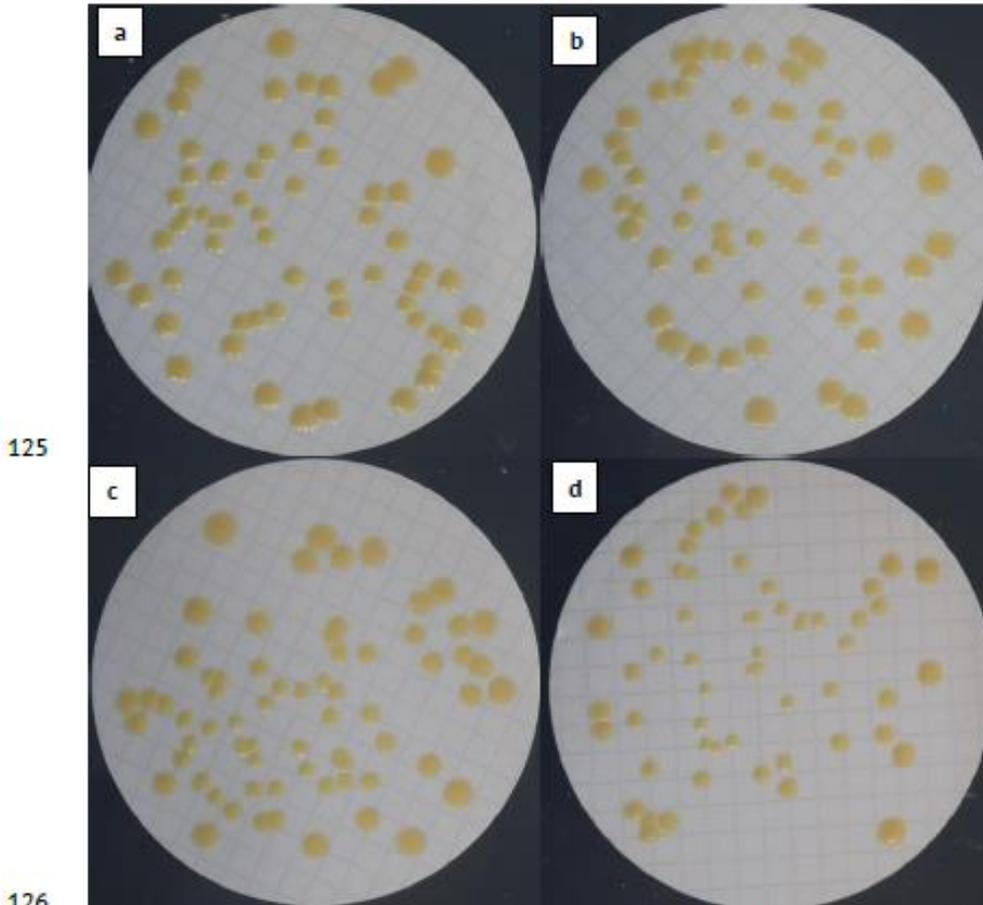
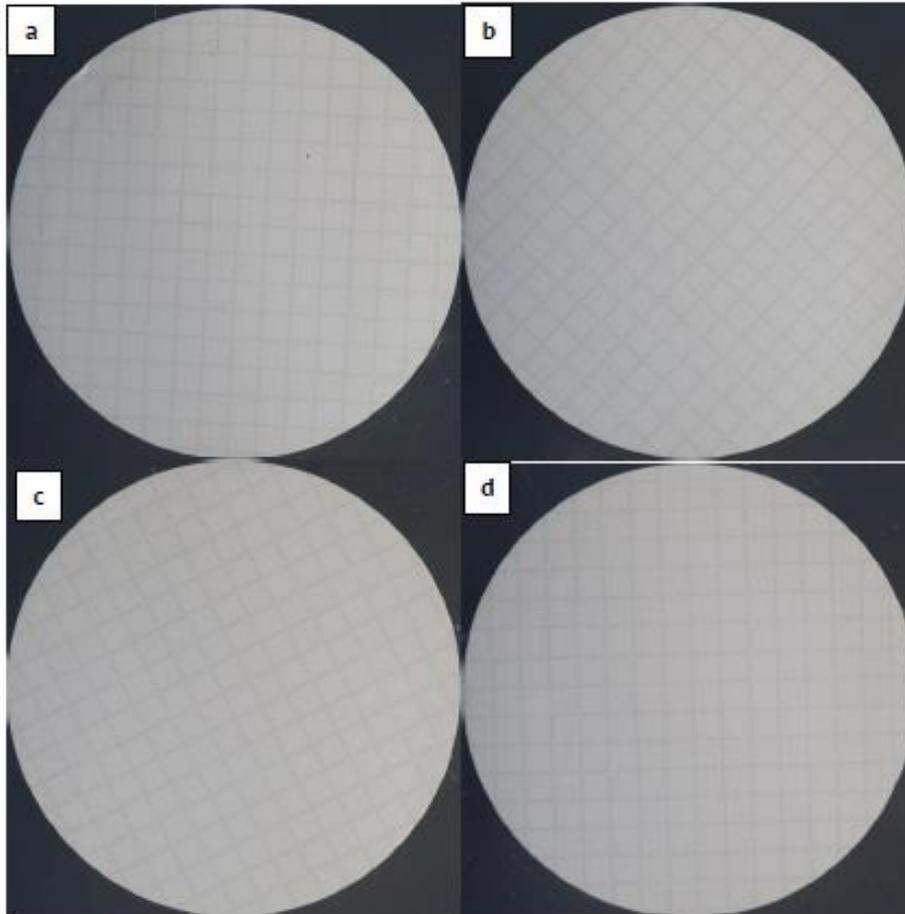


Figure 3. Viability of the production strain spiked in the test material from batches 1 (filter a), 2 (filter b) and 3 (filter c). Filter d represents positive control (production strain but no test material). Incubation at 30 °C, five days.

4.3 Analysis of non-spiked test material: Presence of viable production strain cells or background contamination in the test material

The outcome of the presence of the production strain or contamination in the test material is shown in Figures 4 to 6 and in Table 1. No colonies were detected in 9 g of test material. No colonies were detected in the negative control (Figures 4 to 6).

Daesang: D-psicose (synonymous with D-allulose)



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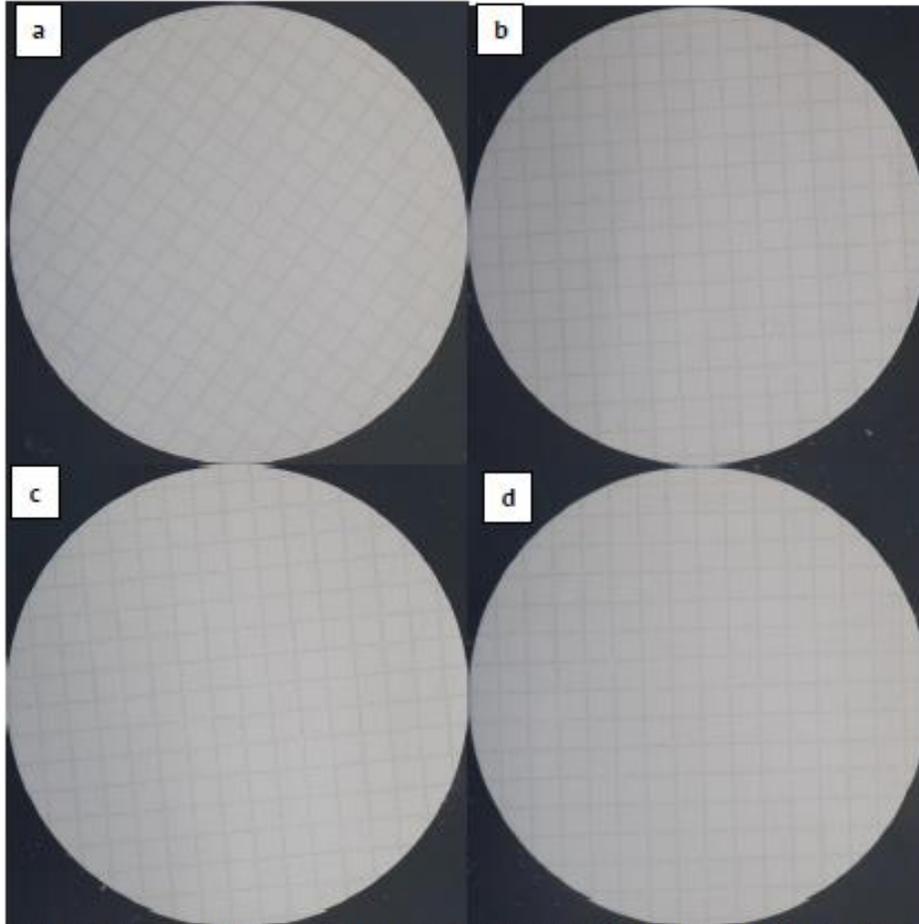
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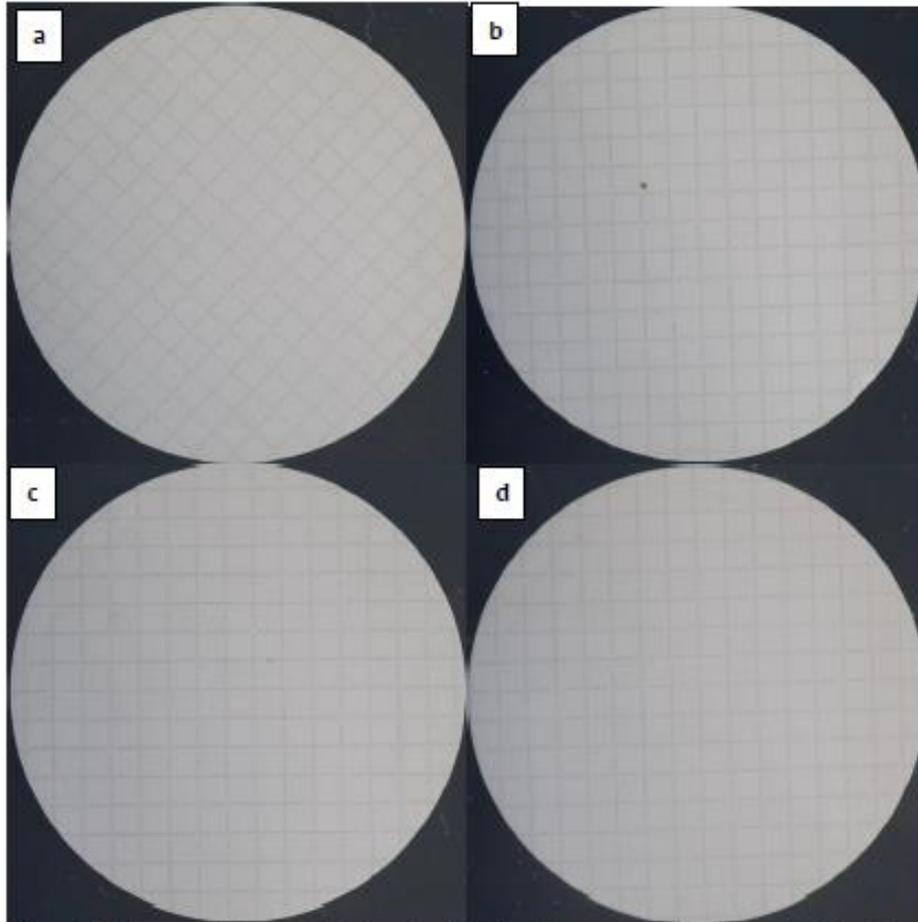
Figure 4. Absence of the production strain in the three non-spiked samples from the test material batch 1 (filters a-c, corresponding to 1 g of test material each) and in the negative control (filter d) incubated at 30 °C for five days. No colonies were detected.



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Figure 5. Absence of the production strain in the three non-spiked samples from the test material batch 2 (filters a-c, corresponding to 1 g of test material each) and in the negative control (filter d) incubated at 30 °C for five days. No colonies were detected.



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148 **Figure 6.** Absence of the production strain in the three non-spiked samples from the test material batch
149 3 (filters a-c, corresponding to 1 g of test material each) and in the negative control (filter d) incubated
150 at 30 °C for five days. No colonies were detected.

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Table 1. Microbial count in 9 g of test material.

The number of colonies in the three batches, three samples from each batch, is shown.

Batch	Sample A, CFU/filter	Sample B, CFU/filter	Sample C, CFU/filter	Total CFU / 3 filters
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0

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5 CONCLUSIONS

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The absence of viable cells of the production strain was demonstrated in nine samples, three from each of the three independent manufacturing batches. Each of the nine samples of 1 g was taken from 10-g subsamples. Altogether, 9 g of test material were analysed. No production strain colonies were detected.

158 REFERENCES

- 159 EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen
160 G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G,
161 Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M,
162 Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R,
163 Galobart J, 2018. Guidance on the characterisation of microorganisms used as feed additives or as
164 production organisms. EFSA Journal, 16(3), 5206, 24 pp. <https://doi.org/10.2903/j.efsa.2018.5206>
165

158 REFERENCES

- 159 EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen
160 G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G,
161 Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M,
162 Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R,
163 Galobart J, 2018. Guidance on the characterisation of microorganisms used as feed additives or as
164 production organisms. EFSA Journal, 16(3), 5206, 24 pp. <https://doi.org/10.2903/j.efsa.2018.5206>
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Daesang: D-psicose (synonymous with D-allulose)

Appendix C. NHANES FOOD CODES

Comparison of food codes reported in NHANES 2007-2010 (0710), 2011-2014(1114), and 2017-2018 (1718).

This appendix compares the food codes listed in 2007-2010, 2011-2014, and 2017-2018 NHANES datasets. Many food codes listed in the NHANES 2007-2010 and 2011-2014 dataset have been dropped and are no longer available in the 2015-2018 NHANES dataset. A significant part of the drop was accounted for by 2 of the 22 food groups targeted for intended use of D-psicose. Group 2 ‘Beverages, carbonated, low calorie’ and group 22 ‘Alcoholic beverages (pre-mixed cocktails, wine coolers, and malt beverages) (low/reduced kcal only)’.

0710	1114	1718		Bakery products (rolls, cakes, pies, pastries, and cookies) rolls, cakes, pastries, cakes, low calorie or dietetic (proposed usage = 10%)
X	X		51152000	Roll, white, soft, reduced calorie and/or high fiber
X	X		51152100	Roll, white, soft, reduced calorie and/or high fiber, toasted
X	X	X	51154510	Roll, diet
X			51165100	Coffee cake, yeast type, fat free, cholesterol free, with fruit
X			53102300	Cake, applesauce, diet, without icing
X	X	X	53104300	Cake, carrot, diet
X			53104520	Cheesecake, diet
X			53104570	Cheesecake, diet, with fruit
X			53104650	Cheesecake, chocolate, reduced fat
X	X	X	53105500	Cake, chocolate, with icing, diet
X			53105600	Cake, chocolate, devil's food, or fudge, pudding-type mix, made by "Lite" recipe (eggs and water added to dry mix, no oil added to dry mix), with icing, coating, or filling
	X	X	53108220	Snack cake, chocolate, with icing or filling, reduced fat and calories
X			53109210	Cake, cupcake, not chocolate, with icing or filling, lowfat, cholesterol free
	X	X	53109220	Snack cake, not chocolate, with icing or filling, reduced fat and calories
X			53109270	Cake, cupcake, chocolate, with or without icing, fruit filling or cream filling, lowfat, cholesterol free
X			53115500	Cake, pineapple, fat free, cholesterol free, without icing
X			53116280	Cake, pound, chocolate, fat free, cholesterol free
X			53116380	Cake, pound, fat free, cholesterol free
X	X	X	53116390	Cake, pound, reduced fat, cholesterol free
X			53120400	Cake, white, eggless, lowfat

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	53420210	Cream puff, eclair, custard or cream filled, iced, reduced fat
X			53511500	Danish pastry, with cheese, fat free, cholesterol free
X	X	X	53530010	Breakfast tart, lowfat
X			53610120	Coffee cake, crumb or quick-bread type, reduced fat, cholesterol free
			Beverages, carbonated, low calorie (proposed usage = 4%)	
X	X	X	92400100	Soft drink, NFS, diet
X	X	X	92410320	Soft drink, cola, diet
X	X	X	92410350	Soft drink, cola, decaffeinated, diet
X	X	X	92410370	Soft drink, pepper type, diet
X	X	X	92410400	Soft drink, pepper type, decaffeinated, diet
X	X	X	92410420	Soft drink, cream soda, diet
X	X	X	92410520	Soft drink, fruit flavored, diet, caffeine free
X	X	X	92410560	Soft drink, fruit flavored, caffeine containing, diet
X	X	X	92410620	Soft drink, ginger ale, diet
X	X	X	92410720	Soft drink, root beer, diet
X	X	X	92410820	Soft drink, chocolate flavored, diet
X	X	X	92411610	Soft drink, cola, fruit or vanilla flavored, diet
X	X	X	92411620	Soft drink, cola, chocolate flavored, diet
			Beverages non-carbonated (non-alcoholic, low calorie, reduced calorie, sugar-free) (proposed usage = 4%)	
X			11613000	Instant breakfast, powder, sweetened with low calorie sweetener, milk added
X			28401200	Gelatin drink, powder, flavored, with low-calorie sweetener, reconstituted
	X	X	92513010	Slush frozen drink, no sugar added
X	X	X	92550030	Fruit juice drink, with high vitamin C, light
	X	X	92550035	Fruit juice drink, light
X	X	X	92550040	Fruit juice drink, diet
X	X	X	92550110	Cranberry juice drink, with high vitamin C, light
	X	X	92550200	Grape juice drink, light
X	X	X	92550350	Orange juice beverage, 40-50% juice, light
	X	X	92550360	Apple juice beverage, 40-50% juice, light
	X	X	92550370	Lemonade, fruit juice drink, light
	X	X	92550380	Pomegranate juice beverage, 40-50% juice, light
X	X	X	92550400	Vegetable and fruit juice drink, with high vitamin C, diet
X	X	X	92550405	Vegetable and fruit juice drink, with high vitamin C, light
X	X	X	92550610	Fruit flavored drink, with high vitamin C, diet
X	X	X	92550620	Fruit flavored drink, diet
X	X	X	92552000	Fruit flavored drink, with high vitamin C, powdered, reconstituted, diet
X	X	X	92552010	Fruit flavored drink, powdered, reconstituted, diet

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	92552020	Fruit juice drink, reduced sugar (Sunny D)
X			92553000	Fruit-flavored thirst quencher beverage, low calorie
X			92565000	Fruit-flavored sports drink or thirst quencher beverage, low calorie
X			92565100	Gatorade G2 thirst quencher sports drink, low calorie
X			92565200	Powerade Zero sports drink, low calorie
X			92582120	Fruit flavored drink, reduced sugar, with high vitamin C, plus added calcium
X			92650005	Red Bull Energy Drink, sugar-free
X			92650210	Mountain Dew AMP Energy Drink, sugar-free
X			92650705	Rockstar Energy Drink, sugar-free
X			92650805	Vault Zero Energy drink
	X	X	95312400	Energy drink, low calorie (Monster)
		X	95312410	Energy drink, sugar free (Monster)
	X	X	95312500	Energy drink, sugar free (Mountain Dew AMP)
	X	X	95312550	Energy drink, sugar free (No Fear)
	X	X	95312555	Energy drink, sugar-free (NOS)
	X	X	95312600	Energy drink, sugar-free (Red Bull)
	X	X	95312700	Energy drink, sugar free (Rockstar)
	X	X	95312800	Energy drink, sugar free (Vault)
	X	X	95313200	Energy drink, sugar free
	X	X	95322200	Sports drink, low calorie (Gatorade G2)
	X	X	95322500	Sports drink, low calorie (Powerade Zero)
	X	X	95323000	Sports drink, low calorie
	X		95341000	FUZE Slenderize fortified low calorie fruit juice beverage
				Cereals, RTE, < 5% added sugar (proposed usage = 10%)
X	X	X	57000100	Cereal, oat, NFS
X	X	X	57123000	Cereal (General Mills Cheerios)
X	X	X	57206700	Cereal (General Mills Fiber One)
X	X	X	57230000	Cereal (Post Grape-Nuts)
X	X	X	57301500	Cereal (Kashi 7 Whole Grain Puffs)
X	X	X	57306700	Cereal (Malt-O-Meal Toasted Oat Cereal)
X	X		57307500	Cereal, millet, puffed
X	X	X	57340000	Cereal, puffed rice
X	X		57341000	Cereal (Post Shredded Wheat'n Bran)
X	X	X	57401100	Cereal, toasted oat
X	X	X	57408100	Cereal (Uncle Sam)
X	X		57410000	Cereal (Weetabix Whole Grain)
X	X	X	57416000	Cereal, puffed wheat, plain

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	57417000	Cereal (Post Shredded Wheat)
				Cereals, low and reduced calorie, sugar free (proposed usage = 5%)
X	X		57000000	Cereal, NFS
X	X		57000050	Kashi cereal, NS as to ready to eat or cooked
X	X	X	57101000	Cereal (Kellogg's All-Bran)
X			57101020	All-Bran with Extra Fiber
X	X		57102000	Cereal (Alpen)
X	X	X	57103000	Cereal (Post Alpha-Bits)
X	X		57103050	Amaranth Flakes
X	X	X	57106050	Cereal (Post Great Grains Banana Nut Crunch)
X	X		57106250	Cereal (General Mills Kix Berry Berry)
X	X		57111000	Bran Chex
X	X		57124000	Chex cereal, NFS
X			57128880	Complete Oat Bran Flakes, Kellogg's (formerly Common Sense Oat Bran, plain)
X	X		57131000	Cereal (Quaker Corn Bran Crunch)
X	X	X	57132000	Cereal (General Mills Chex Corn)
X	X	X	57134000	Cereal, corn flakes
X	X		57134090	Corn flakes, low sodium
X	X	X	57135000	Cereal (Kellogg's Corn Flakes)
X	X	X	57137000	Cereal, corn puffs
X			57138000	Total Corn Flakes
X	X	X	57148000	Cereal (Kellogg's Crispix)
X	X		57148500	Cereal, crispy brown rice
X	X	X	57151000	Cereal, crispy rice
X			57160000	Curves Fruit and Nut Crunch Cereal
X	X		57201900	Cereal (General Mills Dora the Explorer)
			57206705	Cereal (General Mills Fiber One Caramel Delight)
		X	57206710	Cereal (General Mills Fiber One Honey Clusters)
X	X		57206800	Cereal (Health Valley Fiber 7 Flakes)
X	X	X	57207000	Cereal, bran flakes
X	X	X	57208000	Cereal (Kellogg's All-Bran Complete Wheat Flakes)
X	X	X	57209000	Cereal (Post Bran Flakes)
X			57213900	Frosted Chex
X	X	X	57214000	Cereal (Kellogg's Frosted Mini-Wheats)
X	X	X	57228000	Granola, homemade
X	X		57231000	Cereal (Post Grape-Nuts Flakes)
X	X		57231250	Cereal (Post Great Grains Double Pecan Whole Grain Cereal)

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	57237100	Cereal (Post Honey Bunches of Oats Honey Roasted)
X	X	X	57237200	Cereal (Post Honey Bunches of Oats with Vanilla Bunches)
X	X	X	57237300	Cereal (Post Honey Bunches of Oats with Almonds)
		X	57237900	Cereal (Post Honey Bunches of Oats Just Bunches)
X	X	X	57241200	Cereal (Post Shredded Wheat Honey Nut)
X			57243870	Jenny O's
X			57244000	Just Right
X			57301100	Kaboom
X	X	X	57301505	Cereal (Kashi Autumn Wheat)
X	X	X	57301510	Cereal (Kashi GOLEAN)
X	X		57301520	Cereal (Kashi Good Friends)
X	X	X	57301530	Cereal (Kashi Heart to Heart Honey Toasted Oat)
		X	57301540	Cereal (Kashi Honey Sunshine Squares)
X	X		57302100	Cereal (Quaker King Vitaman)
X	X	X	57303100	Cereal (General Mills Kix)
		X	57303105	Cereal (General Mills Honey Kix)
X	X	X	57304100	Cereal (Quaker Life)
X	X		57305200	Cereal (Malt-O-Meal Crispy Rice)
		X	57305215	Cereal (Malt-O-Meal Frosted Mini Spooners)
X	X		57306100	Malt-O-Meal Puffed Rice
X	X		57306120	Malt-O-Meal Puffed Wheat
X	X		57307600	Mini-Swirlz Cinnamon Bun Cereal, Kellogg's
X	X		57308300	Multi Bran Chex
X	X	X	57308400	Cereal (General Mills Cheerios Multigrain)
X			57308900	Natural Muesli, Jenny's Cuisine
X			57311700	Nu System Cuisine Toasted Grain Circles
X	X		57316200	Cereal, nutty nuggets
X	X		57319000	100% Natural Cereal, plain, Quaker
X	X		57319500	Sun Country 100% Natural Granola, with Almonds
X	X		57321800	Optimum Slim, Nature's Path
		X	57321900	Cereal (Nature's Path Organic Flax Plus)
		X	57321905	Organic Flax Plus, Pumpkin Granola, Nature's Path
X	X		57325000	Cereal (Kellogg's Product 19)
		X	57326000	Cereal (Barbara's Puffins)
X	X	X	57327450	Cereal (Quaker Toasted Oat Bran)
X	X	X	57327500	Cereal (Quaker Oatmeal Squares)
X	X	X	57336000	Cereal (General Mills Chex Rice)

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	57337000	Cereal, rice flakes
X	X	X	57339000	Cereal (Kellogg's Rice Krispies)
X	X	X	57344000	Cereal (Kellogg's Special K)
X	X		57403100	Toasties, Post
X			57404100	Malt-O-Meal Toasty O's
X	X		57406100	Cereal (General Mills Total)
X	X	X	57411000	Cereal (General Mills Chex Wheat)
X	X	X	57418000	Cereal (General Mills Wheaties)
Cereals, regular (proposed usage = 2%)				
X	X	X	57100100	Cereal, ready-to-eat, NFS
X			57100400	Character cereals, TV or movie, General Mills
X			57100500	Character cereals, TV or movie, Kellogg's
X	X		57103020	Alpha-bits with marshmallows
X	X	X	57103100	Cereal (General Mills Cheerios Apple Cinnamon)
X			57103500	Apple Cinnamon Squares Mini-Wheats, Kellogg's (formerly Apple Cinnamon Squares)
X	X	X	57104000	Cereal (Kellogg's Apple Jacks)
	X	X	57106060	Cereal (General Mills Cheerios Banana Nut)
X	X		57106100	Cereal (General Mills Basic 4)
X	X	X	57106260	Cereal (General Mills Cheerios Berry Burst)
X	X		57106530	Cereal (Post Selects Blueberry Morning)
X	X		57107000	Cereal (General Mills Boo Berry)
X	X		57110000	Cereal (Kellogg's All-Bran Bran Buds)
X	X	X	57117000	Cereal (Quaker Cap'n Crunch)
X	X	X	57117500	Cereal (Quaker Christmas Crunch)
X	X	X	57119000	Cereal (Quaker Cap'n Crunch's Crunchberries)
X	X	X	57120000	Cereal (Quaker Cap'n Crunch's Peanut Butter Crunch)
	X	X	57124030	Cereal (General Mills Chex Chocolate)
	X	X	57124050	Cereal (General Mills Chex Cinnamon)
	X	X	57124100	Cereal (General Mills Cheerios Chocolate)
X	X	X	57124200	Cereal, chocolate flavored, frosted, puffed corn
X	X	X	57124300	Cereal (General Mills Lucky Charms Chocolate)
X			57124500	Cinnamon Grahams, General Mills
	X		57124900	Cereal (Kellogg's Cinnabon)
X	X	X	57125000	Cereal (General Mills Cinnamon Toast Crunch)
X	X	X	57125010	Cereal (General Mills 25% Less Sugar Cinnamon Toast Crunch)
X	X	X	57125900	Cereal (General Mills Honey Nut Clusters)
X	X	X	57126000	Cereal (Kellogg's Cocoa Krispies)

Daesang: D-psicose (synonymous with D-allulose)

X			57126500	Cocoa Blasts, Quaker
X	X	X	57127000	Cereal (Post Cocoa Pebbles)
X	X	X	57128000	Cereal (General Mills Cocoa Puffs)
		X	57128005	Cereal (General Mills 25% Less Sugar Cocoa Puffs)
X	X	X	57130000	Cereal (General Mills Cookie Crisp)
X	X	X	57139000	Cereal (General Mills Count Chocula)
X	X	X	57143000	Cereal (Kellogg's Cracklin' Oat Bran)
X	X	X	57143500	Cereal (Post Great Grains, Cranberry Almond Crunch)
X	X		57144000	Crisp Crunch
X			57148600	Harmony cereal, General Mills
X			57152000	Crispy Wheats'n Raisins
X			57201800	Disney cereals, Kellogg's
X	X		57206000	Cereal (Famila)
	X	X	57206715	Cereal (General Mills Fiber One Raisin Bran Clusters)
X	X	X	57211000	Cereal (General Mills Frankenberry)
X			57212100	French Toast Crunch, General Mills
X	X	X	57213000	Cereal (Kellogg's Froot Loops)
X			57213005	Froot Loops Cereal Straws
	X	X	57213010	Cereal (Kellogg's Froot Loops Marshmallow)
X	X	X	57213850	Cereal (General Mills Cheerios Frosted)
X	X		57214100	Frosted Wheat Bites
X	X		57215000	Frosty O's
X	X	X	57216000	Cereal, frosted rice
X	X		57218000	Cereal (Kellogg's Frosted Krispies)
X	X		57219000	Cereal, fruit and fiber
X	X		57221000	Cereal, fiber and fruit
X	X		57221650	Fruit Harvest cereal, Kellogg's
X	X	X	57221700	Cereal, fruit rings
X	X		57221800	Cereal, fruit whirls
X	X	X	57221810	Cereal (General Mills Cheerios Fruity)
X	X	X	57223000	Cereal (Post Fruity Pebbles)
X	X	X	57224000	Cereal (General Mills Golden Grahams)
X	X	X	57227000	Cereal, granola
X	X	X	57229000	Cereal (Kellogg's Low Fat Granola)
X	X		57229500	Cereal (Kellogg's Low Fat Granola with Raisins)
			57231100	Grape-Nuts Trail Mix Crunch
X	X	X	57231200	Cereal (Post Great Grains Raisins, Dates, and Pecans)

Daesang: D-psicose (synonymous with D-allulose)

	X		57237310	Cereal (Post Honey Bunches of Oats wth Pecan Bunches)
X	X	X	57238000	Cereal (Post Honeycomb)
X	X		57239000	Honeycomb, strawberry
X	X		57239100	Cereal (Kellogg's Honey Crunch Corn Flakes)
X	X	X	57240100	Cereal (General Mills Chex Honey Nut)
X	X	X	57241000	Cereal (General Mills Cheerios Honey Nut)
X	X	X	57243000	Cereal (Kellogg's Honey Smacks)
X			57245000	Just Right Fruit and Nut (formerly Just Right with raisins, dates, and nuts)
X	X	X	57301511	Cereal (Kashi GOLEAN Crunch)
X	X	X	57301512	Cereal (Kashi GOLEAN Crunch Honey Almond Flax)
		X	57301535	Cereal (Kashi Heart to Heart Oat Flakes and Blueberry Clusters)
		X	57303200	Cereal (Kellogg's Krave)
X	X	X	57305100	Cereal (General Mills Lucky Charms)
X	X	X	57305150	Cereal, frosted oat cereal with marshmallows
		X	57305160	Cereal (Malt-O-Meal Blueberry Muffin Tops)
		X	57305165	Cereal (Malt-O-Meal Cinnamon Toasters)
X	X	X	57305170	Cereal (Malt-O-Meal Coco-Roos)
		X	57305174	Cereal (Malt-O-Meal Colossal Crunch)
		X	57305175	Cereal (Malt-O-Meal Cocoa Dyno-Bites)
X	X	X	57305180	Cereal (Malt-O-Meal Corn Bursts)
X	X	X	57305210	Cereal (Malt-O-Meal Frosted Flakes)
X	X	X	57305300	Cereal (Malt-O-Meal Fruity Dyno-Bites)
		X	57305400	Cereal (Malt-O-Meal Honey Graham Squares)
X	X	X	57305500	Cereal (Malt-O-Meal Honey Nut Toasty O's)
X	X	X	57305600	Cereal (Malt-O-Meal Marshmallow Mateys)
		X	57306130	Cereal (Malt-O-Meal Raisin Bran)
X	X	X	57306500	Cereal (Malt-O-Meal Golden Puffs)
X	X	X	57306800	Cereal (Malt-O-Meal Tootie Fruities)
X	X		57307010	Cereal (Post Maple Pecan Crunch)
X			57307150	Marshmallow Safari, Quaker
X	X		57308150	Mueslix cereal, NFS
X	X	X	57308190	Cereal, muesli
X	X	X	57309100	Cereal (Nature Valley Granola)
X	X		57316300	Cereal (Health Valley Oat Bran Flakes)
		X	57316380	Cereal (General Mills Cheerios Oat Cluster Crunch)
		X	57316385	Cereal (General Mills Cheerios Protein)
X			57316410	Oatmeal Crisp, Apple Cinnamon (formerly Oatmeal Crisp with Apples)

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	57316450	Cereal (General Mills Oatmeal Crisp with Almonds)
X	X		57316500	Cereal (General Mills Oatmeal Crisp with Raisins)
X	X	X	57316710	Cereal (Quaker Honey Graham Oh's)
X			57316750	Oh's, Fruitangy, Quaker
X			57318000	100% Bran
X	X	X	57320500	Cereal (Quaker Granola with Oats, Honey, and Raisins)
X	X		57321500	100 % Natural Wholegrain Cereal with raisins, lowfat, Quaker
X	X		57321700	Optimum, Nature's Path
X			57322500	Oreo O's cereal, Post
X	X		57323000	Cereal (Quaker Sweet Crunch)
X			57323050	Sweet Puffs, Quaker
X			57324000	Peanut Butter Toast Crunch, General Mills
X	X		57328000	Cereal (Quaker Quisp)
X	X	X	57329000	Cereal, raisin bran
X	X	X	57330000	Cereal (Kellogg's Raisin Bran)
X	X	X	57330010	Cereal (Kellogg's Raisin Bran Crunch)
X	X	X	57331000	Cereal (Post Raisin Bran)
X	X		57332050	Cereal (General Mills Total Raisin Bran)
X	X	X	57332100	Cereal (General Mills Raisin Nut Bran)
X	X	X	57335550	Cereal (General Mills Reese's Puffs)
X			57339100	Rice Krispies with Real Strawberries, Kellogg's
X	X	X	57339500	Cereal (Kellogg's Rice Krispies Treats Cereal)
X	X	X	57341200	Cereal (Kellogg's Smart Start Strong)
		X	57341300	Cereal (Kellogg's Smorz)
X	X		57342010	Smorz, Kellogg's
	X	X	57344001	Cereal (Kellogg's Special K Blueberry)
X	X	X	57344005	Cereal (Kellogg's Special K Chocolatey Delight)
	X		57344007	Cereal (Kellogg's Special K Low Fat Granola)
X	X	X	57344010	Cereal (Kellogg's Special K Red Berries)
X	X	X	57344015	Cereal (Kellogg's Special K Fruit & Yogurt)
X	X	X	57344020	Cereal (Kellogg's Special K Vanilla Almond)
X	X	X	57344025	Cereal (Kellogg's Special K Cinnamon Pecan)
X	X		57346500	Oatmeal Honey Nut Heaven, Quaker (formerly Toasted Oatmeal, Honey Nut)
X	X	X	57347000	Cereal (Kellogg's Corn Pops)
X	X	X	57348000	Cereal, frosted corn flakes
X	X	X	57349000	Cereal (Kellogg's Frosted Flakes)
X	X		57349020	Cereal (Kellogg's Frosted Flakes, Reduced Sugar)

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	57355000	Cereal (Post Golden Crisp)
X			57404200	Malt-O-Meal Apple and Cinnamon Toasty O's
X	X		57406105	Total Cranberry Crunch
X	X	X	57407100	Cereal (General Mills Trix)
X	X		57407110	Cereal (General Mills 25% Less Sugar Trix)
X	X		57409100	Cereal (Post Waffle Crisp)
X	X		57413000	Wheat germ, with sugar and honey
X	X	X	57416010	Cereal, puffed wheat, sweetened
X	X		57419000	Cereal (General Mills Cheerios Yogurt Burst)
				Chewing gum (proposed usage = 50%)
X	X	X	91800100	Chewing gum, NFS
X	X	X	91801000	Chewing gum, regular
X	X	X	91802000	Chewing gum, sugar free
				Coffee mix (proposed usage = 30%)
X	X	X	92103000	Coffee, instant, reconstituted
X	X	X	92104000	Coffee, instant, 50% less caffeine, reconstituted
X	X		92106000	Coffee, acid neutralized, from powdered instant
X	X	X	92114000	Coffee, instant, decaffeinated, reconstituted
X	X	X	92121000	Coffee, instant, pre-lightened and pre-sweetened with sugar, reconstituted
	X	X	92121001	Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with sugar, reconstituted
X	X	X	92121010	Coffee, instant, pre-sweetened with sugar, reconstituted
X	X	X	92121020	Coffee, mocha, instant, pre-lightened and pre-sweetened with sugar, reconstituted
X	X	X	92121030	Coffee, mocha, instant, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted
X	X	X	92121040	Coffee, instant, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted
	X	X	92121041	Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted
X	X	X	92121050	Coffee, mocha, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, reconstituted
X	X		92191000	Coffee, dry instant powder, NS as to regular or decaffeinated
X	X	X	92191100	Coffee, instant, not reconstituted
	X	X	92191105	Coffee, instant, 50% less caffeine, not reconstituted
X	X	X	92191200	Coffee, instant, decaffeinated, not reconstituted
X	X		92191250	Coffee, dry, acid neutralized
	X	X	92191400	Coffee, instant, pre-sweetened with sugar, not reconstituted
X	X		92191500	Coffee and chicory, dry instant powder
X	X		92191520	Coffee, decaffeinated, and chicory, dry instant powder
X	X	X	92192000	Coffee, mocha, instant, pre-lightened and pre-sweetened with sugar, not reconstituted
X	X	X	92192030	Coffee, mocha, instant, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	92192040	Coffee, mocha, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted
X	X	X	92193000	Coffee, instant, pre-lightened and pre-sweetened with sugar, not reconstituted
	X	X	92193005	Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with sugar, not reconstituted
X	X	X	92193020	Coffee, instant, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted
	X	X	92193025	Coffee, instant, decaffeinated, pre-lightened and pre-sweetened with low calorie sweetener, not reconstituted
Confections and frostings (proposed usage = 5%)				
X	X		44101000	Carob powder or flour
X	X	X	44201000	Carob chips
X	X	X	91700010	Candy, NFS
X	X	X	91700500	M&M's Almond Chocolate Candies
X	X	X	91701010	Almonds, chocolate covered
X	X	X	91701020	Almonds, sugar-coated
X	X	X	91701030	Almonds, yogurt-covered
X	X	X	91702010	Butterscotch morsels
X	X	X	91703010	Caramel, chocolate-flavored roll
X	X	X	91703020	Caramel, flavor other than chocolate
X	X	X	91703030	Caramel, with nuts
X	X	X	91703040	Caramel candy, chocolate covered
X	X	X	91703050	Caramel with nuts and cereal, chocolate covered
X	X	X	91703060	Caramel with nuts, chocolate covered
X	X	X	91703070	Rolo
X	X	X	91703150	Toblerone, milk chocolate with honey and almond nougat
X	X	X	91703200	TWIX Caramel Cookie Bars
X	X	X	91703250	TWIX Chocolate Fudge Cookie Bars
X	X	X	91703300	TWIX Peanut Butter Cookie Bars
X	X	X	91703400	Whatchamacallit
X	X	X	91703500	Nuts, carob-coated
X	X	X	91703600	Espresso coffee beans, chocolate-covered
X	X	X	91705010	Milk chocolate candy, plain
X	X	X	91705020	Milk chocolate candy, with cereal
X	X	X	91705030	Kit Kat
X	X	X	91705040	Chocolate, milk, with nuts, not almond or peanuts
X	X	X	91705050	Milk chocolate candy, with fruit and nuts
X	X	X	91705060	Milk chocolate candy, with almonds
X	X	X	91705070	Chocolate, milk, with peanuts
X	X	X	91705090	Chocolate candy with fondant and caramel

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	91705200	Chocolate, semi-sweet morsel
X	X	X	91705300	Chocolate, sweet or dark
	X	X	91705310	Chocolate, sweet or dark, with almonds
X	X	X	91705400	Chocolate, white
X	X	X	91705410	Chocolate, white, with almonds
X	X	X	91705420	Chocolate, white, with cereal
	X	X	91705430	Kit Kat White
X	X	X	91705500	Mexican chocolate, tablet
X	X	X	91706000	Coconut candy, chocolate covered
X	X	X	91706100	Coconut candy, no chocolate covering
X	X	X	91706400	Coconut candy, Puerto Rican style
X	X	X	91707000	Fondant
X	X	X	91707010	Fondant, chocolate covered
X	X	X	91708000	Fruit peel, candied
X	X	X	91708010	Date candy
X	X	X	91708020	Soft fruit confections
X	X	X	91708040	Fun Fruits Creme Supremes
X	X	X	91708070	Tamarind candy
X	X	X	91708150	Yogurt covered fruit snacks candy, with added vitamin C
X	X	X	91708160	Yogurt covered fruit snacks candy rolls, with high vitamin C
X	X	X	91709000	Gumdrops, chocolate covered
X	X	X	91713010	Fudge, chocolate, chocolate-coated
X	X	X	91713020	Fudge, chocolate, chocolate-coated, with nuts
X	X	X	91713030	Fudge, chocolate
X	X	X	91713040	Fudge, chocolate, with nuts
X	X	X	91713050	Fudge, peanut butter
X	X	X	91713060	Fudge, peanut butter, with nuts
X	X	X	91713070	Fudge, vanilla
X	X	X	91713080	Fudge, vanilla, with nuts
X	X	X	91713090	Fudge, divinity
X	X	X	91713100	Fudge, brown sugar, penuche
X	X	X	91715000	Fudge, caramel and nut, chocolate-coated candy
X	X	X	91715100	SNICKERS Bar
X	X	X	91715200	Baby Ruth
X	X	X	91715300	100 GRAND Bar
X	X	X	91716010	Halvah, plain
X	X	X	91716110	Halvah, chocolate covered

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	91718100	Butterfinger
	X	X	91718110	Butterfinger Crisp
X	X	X	91718200	Chocolate-flavored sprinkles
X	X	X	91718300	Ladoo, round ball, Asian-Indian dessert
X	X	X	91721000	Licorice
X	X	X	91723000	Marshmallow
X	X	X	91723010	Marshmallow, chocolate covered
X	X	X	91723020	Marshmallow, candy-coated
X	X		91723050	Marshmallow, coconut-coated
X	X	X	91726000	Nougat, plain
X	X	X	91726110	Nougat, with caramel, chocolate covered
X	X	X	91726130	MILKY WAY Bar
X	X	X	91726140	MILKY WAY MIDNIGHT Bar
X	X	X	91726150	MARS Almond Bar
X	X	X	91726410	Nougat, chocolate covered
X	X	X	91726420	3 MUSKETEERS Bar
	X	X	91726425	3 Musketeers Truffle Crisp Bar
X	X	X	91727010	Nuts, chocolate covered, not almonds or peanuts
X	X	X	91728000	Nut roll, fudge or nougat, caramel and nuts
X	X	X	91728500	Sugared pecans, sugar and egg white coating
X	X	X	91731000	Peanuts, chocolate covered
X	X	X	91731010	M&M's Peanut Chocolate Candies
X	X	X	91731060	M&M's Peanut Butter Chocolate Candies
X	X	X	91731100	Peanuts, sugar-coated
X	X	X	91731150	Peanuts, yogurt covered
X	X	X	91732000	Peanut bar
X	X	X	91732100	Planters Peanut Bar
X	X	X	91733000	Peanut brittle
X	X	X	91733200	Peanut Bar, chocolate covered candy
X	X	X	91734000	Peanut butter, chocolate covered
X	X	X	91734100	Reese's Peanut Butter Cup
X	X	X	91734200	Reese's Pieces
X	X	X	91734300	Reese's Sticks
X	X	X	91734400	Reese's Fast Break
X	X	X	91734450	Reese's Crispy Crunchy Bar
X	X	X	91734500	Peanut butter morsels
X	X	X	91735000	Pralines

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	91736000	Pineapple candy, Puerto Rican style
X	X	X	91739010	Raisins, chocolate covered
X			91739510	Raisins, carob covered
X	X	X	91739600	Raisins, yogurt covered
X	X	X	91742010	Sesame Crunch, Sahadi
X	X	X	91745010	Gumdrops
X	X	X	91745100	Skittles
X	X	X	91746010	Sugar-coated chocolate discs
X	X	X	91746100	M&M's Milk Chocolate Candies
X	X	X	91746120	Sixlets
X	X	X	91746150	Easter egg, candy coated chocolate
	X	X	91746200	M&M's Pretzel Chocolate Candies
X	X	X	91750000	Taffy
X	X	X	91760000	Toffee, plain
X	X	X	91760100	Toffee, chocolate covered
X	X	X	91760200	Toffee, chocolate-coated, with nuts
X	X	X	91760500	Truffles
X	X	X	91760700	Wax candy, liquid filled
				Dressings for salads (proposed usage = 5%)
X	X	X	82101000	Vegetable oil, NFS
X	X	X	82101300	Almond oil
X	X	X	82101500	Coconut oil
X	X	X	82102000	Corn oil
X	X		82102500	Corn and canola oil
X	X	X	82103000	Cottonseed oil
X	X	X	82103500	Flaxseed oil
X	X	X	82104000	Olive oil
X	X	X	82105000	Peanut oil
X	X	X	82105500	Canola oil
X	X		82105750	Canola and soybean oil
X	X		82105800	Canola, soybean and sunflower oil
X	X	X	82106000	Safflower oil
X	X	X	82107000	Sesame oil
X	X	X	82108000	Soybean oil
X	X		82108250	Soybean and sunflower oil
X	X	X	82108500	Sunflower oil
X	X	X	82108700	Walnut oil

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	82109000	Wheat germ oil
X	X	X	83100100	Salad dressing, NFS, for salads
X	X	X	83101000	Blue or roquefort cheese dressing
X	X		83101500	Bacon dressing (hot)
X	X	X	83101600	Bacon and tomato dressing
X	X	X	83102000	Caesar dressing
X	X	X	83103000	Coleslaw dressing
X			83103500	Feta Cheese Dressing
X	X	X	83104000	French or Catalina dressing
X			83105000	Fruit dressing, made with fruit juice and cream
X			83105100	Fruit dressing, made with honey, oil, and water
X	X	X	83105500	Honey mustard dressing
X	X	X	83106000	Italian dressing, made with vinegar and oil
X	X	X	83109000	Russian dressing
X			83111000	Boiled, cooked-type dressing
X	X	X	83112000	Avocado dressing
X	X	X	83112500	Creamy dressing
X			83112600	Cream cheese dressing
X			83112900	Milk, vinegar, and sugar dressing
X	X	X	83112950	Poppy seed dressing
X			83112960	Peppercorn Dressing
X			83112980	Celery seed dressing
X	X	X	83112990	Sesame dressing
X			83113000	Sweet and sour dressing
X	X	X	83114000	Thousand Island dressing
X	X	X	83115000	Yogurt dressing
X	X	X	83200100	Salad dressing, light, NFS
X	X	X	83201000	Blue or roquefort cheese dressing, light
X			83201050	Blue or roquefort cheese dressing, reduced calorie
X			83201200	Blue or roquefort cheese dressing, reduced calorie, fat-free, cholesterol-free
X	X	X	83201400	Coleslaw dressing, light
X			83202000	French dressing, low-calorie
X			83202010	French dressing, reduced calorie, fat-free, cholesterol-free
X	X	X	83202020	French or Catalina dressing, light
X	X	X	83203000	Caesar dressing, light
X	X	X	83204500	Honey mustard dressing, light
X			83205000	Italian dressing, low calorie

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	83205450	Italian dressing, light
X			83205500	Italian dressing, reduced calorie, fat-free
X	X	X	83206000	Russian dressing, light
	X	X	83206500	Sesame dressing, light
X	X	X	83207000	Thousand Island dressing, light
X			83207100	Thousand Island dressing, reduced calorie, fat-free, cholesterol-free
X			83208000	Vinegar, sugar, and water dressing
X	X	X	83208500	Korean dressing or marinade
X			83209000	Milk, vinegar, and artificial sweetener dressing
X			83210000	Creamy dressing, made with sour cream and/or buttermilk and oil, diet, NS as to low or reduced calorie
X			83210050	Creamy dressing made with sour cream and/or buttermilk and oil, low calorie
X	X	X	83210100	Creamy dressing, light
X			83210200	Creamy dressing, made with sour cream and/or buttermilk and oil, reduced calorie, fat-free, cholesterol-free
X			83210250	Creamy dressing, made with sour cream and/or buttermilk and oil, reduced calorie, cholesterol-free
X			83220000	Salad dressing, low calorie, oil-free
	X	X	83300100	Blue or roquefort cheese dressing, fat free
	X	X	83300200	Caesar dressing, fat free
	X	X	83300300	Creamy dressing, fat free
	X	X	83300400	French or Catalina dressing, fat free
	X	X	83300500	Honey mustard dressing, fat free
	X	X	83300600	Italian dressing, fat free
	X	X	83300800	Russian dressing, fat free
	X	X	83300900	Salad dressing, fat free, NFS
	X	X	83301000	Thousand Island dressing, fat free
		X	89901010	Cream sauce, for use with vegetables
			Fat-based cream - used in modified fat/calorie cookies, cakes, pastries and pie (proposed usage = 10%)	
X	X		12110300	Cream, light, whipped, unsweetened
X	X		12130200	Cream, heavy, whipped, unsweetened
X	X	X	12140000	Cream, whipped
X	X		12140100	Cream, whipped, pressurized container
X	X		12140105	Cream, whipped, pressurized container, light
			Frozen dairy desserts (ice cream, soft serve, sorbet: low calorie, reduced calorie, sugar free) (proposed usage = 5%)	
X	X		13110310	Ice cream, no sugar added, NS as to flavor
X	X		13110320	Ice cream, no sugar added, flavors other than chocolate
X	X		13110330	Ice cream, no sugar added, chocolate

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	13130100	Light ice cream, NFS
X	X	X	13130300	Light ice cream, vanilla
X	X	X	13130310	Light ice cream, chocolate
X	X		13130320	Light ice cream, no sugar added, NS as to flavor
X	X		13130330	Light ice cream, no sugar added, flavors other than chocolate
X	X		13130340	Light ice cream, no sugar added, chocolate
X	X		13130590	Light ice cream, soft serve, NS as to flavor
X	X		13130600	Light ice cream, soft serve, flavors other than chocolate
X	X		13130610	Light ice cream, soft serve, chocolate
X	X		13130620	Light ice cream, soft serve cone, flavors other than chocolate
X	X		13130630	Light ice cream, soft serve cone, chocolate
X	X		13130640	Light ice cream, soft serve cone, NS as to flavor
X	X	X	13135000	Light ice cream sandwich, vanilla
X	X	X	13135010	Light ice cream sandwich, chocolate
X	X		13136000	Ice cream sandwich, made with light, no sugar added ice cream
		X	13140000	Light ice cream bar, vanilla
X	X	X	13140100	Light ice cream bar, vanilla, chocolate coated
X	X		13140110	Light ice cream, bar or stick, chocolate covered, with nuts
		X	13140115	Light ice cream bar, chocolate
X	X		13140450	Light ice cream, cone, NFS
X	X		13140500	Light ice cream, cone, flavors other than chocolate
X	X		13140550	Light ice cream, cone, chocolate
		X	13140570	Light ice cream, no sugar added, cone, NS as to flavor
		X	13140575	Light ice cream, no sugar added, cone, flavors other than chocolate
		X	13140580	Light ice cream, no sugar added, cone, chocolate
X	X		13140600	Light ice cream, sundae, soft serve, chocolate or fudge topping, with whipped cream
X	X		13140630	Light ice cream, sundae, soft serve, fruit topping, with whipped cream
X	X		13140650	Light ice cream, sundae, soft serve, not fruit or chocolate topping, with whipped cream
X	X		13140660	Light ice cream, sundae, soft serve, chocolate or fudge topping, without whipped cream
X	X		13140670	Light ice cream, sundae, soft serve, fruit topping, without whipped cream
X	X		13140680	Light ice cream, sundae, soft serve, not fruit or chocolate topping, without whipped cream
		X	13140710	Creamsicle, light
		X	13142100	Light ice cream cone, vanilla, prepackaged
		X	13142110	Light ice cream cone, chocolate, prepackaged
X	X	X	13150000	Sherbet, all flavors
X	X		13160150	Fat free ice cream, no sugar added, chocolate
X	X		13160160	Fat free ice cream, no sugar added, flavors other than chocolate

Daesang: D-psicose (synonymous with D-allulose)

X	X		13160400	Fat free ice cream, flavors other than chocolate
X	X		13160410	Fat free ice cream, chocolate
X	X		13160420	Fat free ice cream, NS as to flavor
X	X		13161000	Milk dessert bar, frozen, made from lowfat milk
X	X		13161500	Milk dessert sandwich bar, frozen, made from lowfat milk
X	X		13161520	Milk dessert sandwich bar, frozen, with low-calorie sweetener, made from lowfat milk
X	X	X	13161600	Fudgesicle, light
X	X		13161630	Light ice cream, bar or stick, with low-calorie sweetener, chocolate coated
Gelatins, pudding, fillings (low calorie, reduced calorie, sugar free) (proposed usage = 10%)				
X	X		13210190	Pudding, Mexican bread, low fat
X	X		13210250	Pudding, chocolate, low calorie, with artificial sweetener, NS as to from dry mix or ready-to-eat
X	X		13210290	Pudding, flavors other than chocolate, low calorie, with artificial sweetener, NS from dry mix or ready-to-eat
X	X	X	13220210	Pudding, flavors other than chocolate, made from dry mix, sugar free
X	X	X	13220220	Pudding, chocolate, made from dry mix, sugar free
X	X		13220230	Pudding, ready-to-eat, chocolate, reduced fat
X	X		13220235	Pudding, ready-to-eat, chocolate, fat free
X	X		13220240	Pudding, ready-to-eat, flavors other than chocolate, reduced fat
X	X		13220245	Pudding, ready-to-eat, flavors other than chocolate, fat free
X	X	X	13230120	Pudding, flavors other than chocolate, ready-to-eat, sugar free
X	X	X	13230140	Pudding, chocolate, ready-to-eat, sugar free
X	X		13230510	Pudding, ready-to-eat, tapioca, fat free
X	X		13250200	Mousse, chocolate, lowfat, reduced calorie, prepared from dry mix, water added
X	X	X	91511010	Gelatin dessert, sugar free
X	X	X	91511020	Gelatin dessert, sugar free, with fruit
X	X		91511030	Gelatin dessert, dietetic, with whipped topping, sweetened with low calorie sweetener
X	X		91511050	Gelatin dessert, dietetic, with cream cheese, sweetened with low calorie sweetener
X	X		91511060	Gelatin dessert, dietetic, with sour cream, sweetened with low calorie sweetener
X	X		91511070	Gelatin dessert, dietetic, with fruit and sour cream, sweetened with low calorie sweetener
X	X		91511080	Gelatin dessert, dietetic, with fruit and cream cheese, sweetened with low calorie sweetener
X	X		91511090	Gelatin dessert, dietetic, with fruits and vegetables, sweetened with low calorie sweetener
X	X		91511100	Gelatin salad, dietetic, with vegetables, sweetened with low calorie sweetener
X	X		91511110	Gelatin dessert, dietetic, with fruit and whipped topping, sweetened with low calorie sweetener
Hard candies (low calorie) (proposed usage = 70%)				
X	X	X	91770020	Dietetic or low calorie hard candy
X	X	X	91770050	Dietetic or low calorie mints
Soft candies (non-chocolate, plain chocolate, chocolate coated) (low calorie, reduced calorie, sugar free) (proposed usage = 25%)				

Daesang: D-psicose (synonymous with D-allulose)

X	X	X	91703080	Caramel, all flavors, sugar free
X	X	X	91770000	Dietetic or low-calorie candy, NFS
X	X	X	91770010	Dietetic or low-calorie gumdrops
X	X	X	91770030	Dietetic or low-calorie candy, chocolate covered
Jams and jellies (proposed usage = 10%)				
X	X	X	91401000	Jelly, all flavors
X	X	X	91402000	Jam, preserve, all flavors
X	X	X	91403000	Fruit butter, all flavors
X	X	X	91404000	Marmalade, all flavors
X	X	X	91405000	Jelly, sugar free, all flavors
X	X	X	91405500	Jelly, reduced sugar, all flavors
X	X	X	91406000	Jam, preserve, marmalade, sugar free, all flavors
X	X	X	91406500	Jam, preserve, marmalade, sweetened with fruit juice concentrates, all flavors
X	X	X	91406600	Jam, preserve, marmalade, reduced sugar, all flavors
Sugar (proposed usage = 10%)				
X	X	X	91101000	Sugar, NFS
X	X	X	91101010	Sugar, white, granulated or lump
X	X	X	91101020	Sugar, white, confectioner's, powdered
X	X	X	91102010	Sugar, brown
X	X		91103010	Sugar, maple
X	X	X	91104100	Sugar, cinnamon
X	X		91104200	Sugar, raw
X	X		91301120	Sugar, caramelized
X	X	X	91302010	Honey
X	X	X	91303000	Molasses
X	X		91303500	Sugar, brown, liquid
Sugar substitutes (proposed usage = 100%)				
X	X		91105010	Fructose sweetener, sugar substitute, dry powder
X	X		91106000	Sugar substitute, sugar-aspartame blend, dry powder
		X	91106010	Sugar substitute and sugar blend
X	X	X	91107000	Sugar substitute, sucralose, powder
X	X	X	91108000	Sugar substitute, stevia, powder
X	X	X	91108010	Sugar substitute, stevia, liquid
		X	91108020	Sugar substitute, monk fruit, powder
		X	91109000	Blue Agave liquid sweetener, sugar substitute
X	X	X	91200000	Sugar substitute, powder, NFS
		X	91200005	Sugar substitute, liquid, NFS

Daesang: D-psicose (synonymous with D-allulose)

X	X		91200020	Sugar substitute, saccharin-based, dry powder
X	X		91200030	Brown sugar substitute, saccharin-based, dry powder
X	X	X	91200040	Sugar substitute, saccharin, powder
X	X	X	91200110	Sugar substitute, saccharin, liquid
X	X	X	91201010	Sugar substitute, aspartame, powder
		X	91302020	Agave liquid sweetener
Sweet sauces and syrups low calorie, reduced calorie and sugar free (proposed usage = 10%)				
X	X		44202000	Carob syrup
X	X	X	91300010	Syrup, NFS
X	X	X	91300100	Pancake syrup
X	X		91301020	Cane and corn pancake syrup
X	X	X	91301030	Corn syrup
X	X		91301040	Buttered blends syrup
X	X	X	91301050	Blueberry syrup
X	X		91301060	Maple syrup
X	X	X	91301080	Chocolate syrup
X	X	X	91301081	Chocolate syrup, light
		X	91301082	Chocolate syrup, thin type, sugar free
X	X		91301090	Sorghum syrup
X	X	X	91301100	Simple syrup
X	X	X	91301130	Strawberry drink syrup
X	X		91301200	Sugar, brown, and water syrup
X	X		91301250	Maple and corn and/or cane pancake syrup blends
X	X	X	91301510	Pancake syrup, light
X	X		91303750	Chocolate gravy
X	X	X	91304010	Topping, butterscotch or caramel
X	X	X	91304020	Topping, chocolate
X	X	X	91304040	Topping, marshmallow
X	X		91304050	Hard sauce
X	X	X	91304060	Topping, nuts and syrup
X	X		91304070	Topping, peanut butter, thick, fudge type
X	X		91304080	Topping, fruit, unsweetened
X	X	X	91304090	Topping, chocolate flavored hazelnut spread
		X	91306020	Caramel dip, regular
		X	91306025	Caramel dip, light
		X	91306030	Chocolate dip
		X	91306040	Dessert dip

Daesang: D-psicose (synonymous with D-allulose)

X	X		91351010	Syrup, dietetic
X	X		91351020	Topping, dietetic
X	X		91361020	Fruit sauce
X			91361030	Raisin sauce
X	X	X	91361040	Dessert sauce
Yogurt (regular and frozen), low calorie, reduced calorie, sugar free (proposed usage = 5%)				
X	X		11422000	Yogurt, vanilla, low fat milk
X	X		11422100	Yogurt, vanilla, low fat milk, light
X	X		11423000	Yogurt, vanilla, nonfat milk
X	X		11424000	Yogurt, vanilla, nonfat milk, light
		X	11424510	Yogurt, Greek, vanilla, low fat
		X	11424520	Yogurt, Greek, vanilla, nonfat
X	X		11427000	Yogurt, chocolate, nonfat milk
		X	11428000	Yogurt, Greek, chocolate, nonfat
X	X	X	11432000	Yogurt, low fat milk, fruit
X	X		11432500	Yogurt, fruit, low fat milk, light
X	X	X	11433000	Yogurt, nonfat milk, fruit
X	X		11433500	Yogurt, fruit, nonfat milk, light
		X	11434010	Yogurt, Greek, low fat milk, fruit
		X	11434020	Yogurt, Greek, nonfat milk, fruit
		X	11434200	Yogurt, low fat milk, flavors other than fruit
		X	11434300	Yogurt, nonfat milk, flavors other than fruit
		X	11435020	Yogurt, Greek, low fat milk, flavors other than fruit
		X	11435030	Yogurt, Greek, nonfat milk, flavors other than fruit
X	X	X	11446000	Yogurt parfait, low fat, with fruit
X	X		11460150	Yogurt, frozen, NS as to flavor, lowfat milk
X	X		11460160	Yogurt, frozen, chocolate, lowfat milk
X	X		11460170	Yogurt, frozen, flavors other than chocolate, lowfat milk
X	X		11460190	Yogurt, frozen, NS as to flavor, nonfat milk
X	X		11460200	Yogurt, frozen, chocolate, nonfat milk
X	X		11460300	Yogurt, frozen, flavors other than chocolate, nonfat milk
X	X		11460400	Yogurt, frozen, chocolate, nonfat milk, with low-calorie sweetener
X	X		11460410	Yogurt, frozen, flavors other than chocolate, nonfat milk, with low-calorie sweetener
X	X		11461270	Yogurt, frozen, cone, flavors other than chocolate, lowfat milk
X	X		11461280	Yogurt, frozen, cone, chocolate, lowfat milk
Nutrition bar, grain based cereal bars, protein bars (proposed usage = 15%)				
X			41435000	Fiber One Fulfill Bar

Daesang: D-psicose (synonymous with D-allulose)

X	41435110	High protein bar, candy-like, soy and milk base
X	41435120	Zone Perfect Classic Crunch nutrition bar
X	41435300	Balance Original Bar
X	41435500	Clif Bar
X	41435700	South Beach Living High Protein Cereal Bar
X	41435710	South Beach Living Meal Replacement Bar
X	53540000	Breakfast bar, NFS
X	53540200	Breakfast bar, cereal crust with fruit filling, lowfat
X	53540300	Fiber One Chewy Bar
X	53540400	Kellogg's Nutri-Grain Cereal Bar
X	53540402	Kellogg's Nutri-Grain Yogurt Bar
X	53540404	Kellogg's Nutri-Grain Fruit and Nut Bar
X	53540500	Breakfast bar, date, with yogurt coating
X	53540600	Milk 'n Cereal bar
X	53540700	Kellogg's Special K bar
X	53540800	Kashi GOLEAN Chewy Bars
X	53540802	Kashi TLC Chewy Granola Bar
X	53540804	Kashi GOLEAN Crunchy Bars
X	53540806	Kashi TLC Crunchy Granola Bar
X	53540900	Nature Valley Chewy Trail Mix Granola Bar
X	53540902	Nature Valley Chewy Granola Bar with Yogurt Coating
X	53540904	Nature Valley Sweet and Salty Nut Granola Bar
X	53540906	Nature Valley Crunchy Granola Bar
X	53541000	Quaker Chewy Granola Bar
X	53541002	Quaker Chewy 90 Calorie Granola Bar
X	53541004	Quaker Chewy 25% Less Sugar Granola Bar
X	53541006	Quaker Chewy Dipps Granola Bar
X	53541200	Meal replacement bar
X	53541300	Slim Fast Original Meal Bar
X	53542000	Snack bar, oatmeal
X	53542100	Granola bar, NFS
X	53542200	Granola bar, lowfat, NFS
X	53542210	Granola bar, nonfat
X	53543000	Granola bar, reduced sugar, NFS
X	53543100	Granola bar, peanuts, oats, sugar, wheat germ
X	53544200	Granola bar, chocolate coated, NFS
X	53544210	Granola bar, with coconut, chocolate-coated

Daesang: D-psicose (synonymous with D-allulose)

X		53544220	Granola bar with nuts, chocolate-coated
X		53544230	Granola bar, oats, nuts, coated with non-chocolate coating
X		53544250	Granola bar, coated with non-chocolate coating
X		53544300	Granola bar, high fiber, coated with non-chocolate yogurt coating
X		53544400	Granola bar, with rice cereal
X		53544410	Quaker Granola Bites
X		53544450	PowerBar (fortified high energy bar)
	X	X	53710400 Cereal or granola bar (General Mills Fiber One Chewy Bar)
	X	X	53710500 Cereal or granola bar (Kellogg's Nutri-Grain Cereal Bar)
	X	X	53710502 Cereal or granola bar (Kellogg's Nutri-Grain Yogurt Bar)
	X	X	53710504 Cereal or granola bar (Kellogg's Nutri-Grain Fruit and Nut Bar)
	X	X	53710600 Milk 'n Cereal bar
	X	X	53710700 Cereal or granola bar (Kellogg's Special K bar)
	X	X	53710800 Cereal or granola bar (Kashi Chewy)
	X	X	53710802 Cereal or granola bar (Kashi Crunchy)
	X		53710804 Kashi GOLEAN Crunchy Bars
	X		53710806 Kashi TLC Crunchy Granola Bar
		X	53710810 Cereal or granola bar (KIND Fruit and Nut Bar)
	X	X	53710900 Cereal or granola bar (General Mills Nature Valley Chewy Trail Mix)
	X	X	53710902 Cereal or granola bar, with yogurt coating (General Mills Nature Valley Chewy Granola Bar)
	X	X	53710904 Cereal or granola bar (General Mills Nature Valley Sweet and Salty Granola Bar)
	X	X	53710906 Cereal or granola bar (General Mills Nature Valley Crunchy Granola Bar)
	X	X	53711000 Cereal or granola bar (Quaker Chewy Granola Bar)
	X	X	53711002 Cereal or granola bar (Quaker Chewy 90 Calorie Granola Bar)
	X	X	53711004 Cereal or granola bar (Quaker Chewy 25% Less Sugar Granola Bar)
	X	X	53711006 Cereal or granola bar (Quaker Chewy Dipp's Granola Bar)
	X	X	53711100 Cereal or granola bar (Quaker Granola Bites)
	X	X	53712000 Snack bar, oatmeal
	X	X	53712100 Cereal or Granola bar, NFS
	X	X	53712200 Cereal or granola bar, lowfat, NFS
	X	X	53712210 Cereal or granola bar, nonfat
	X	X	53713000 Cereal or granola bar, reduced sugar, NFS
		X	53713010 Cereal or granola bar, fruit and nut
	X	X	53713100 Cereal or granola bar, peanuts, oats, sugar, wheat germ
	X	X	53714200 Cereal or granola bar, chocolate coated, NFS
	X	X	53714210 Cereal or granola bar, with coconut, chocolate coated
	X	X	53714220 Cereal or granola bar with nuts, chocolate coated

Daesang: D-psicose (synonymous with D-allulose)

	X	X	53714230	Cereal or granola bar, oats, nuts, coated with non-chocolate coating
	X	X	53714250	Cereal or granola bar, coated with non-chocolate coating
	X	X	53714300	Cereal or granola bar, high fiber, coated with non-chocolate yogurt coating
	X	X	53714400	Cereal or granola bar, with rice cereal
	X	X	53714500	Breakfast bar, NFS
	X	X	53714510	Breakfast bar, date, with yogurt coating
	X	X	53714520	Breakfast bar, cereal crust with fruit filling, lowfat
	X	X	53720100	Nutrition bar (Balance Original Bar)
	X	X	53720200	Nutrition bar (Clif Bar)
	X	X	53720210	Nutrition bar (Clif Kids Organic Zbar)
	X	X	53720300	Nutrition bar (PowerBar)
	X	X	53720400	Nutrition bar (Slim Fast Original Meal Bar)
	X	X	53720500	Nutrition bar (Snickers Marathon Protein Bar)
	X		53720510	Snickers Marathon Energy bar
	X	X	53720600	Nutrition bar (South Beach Living Meal Bar)
	X	X	53720610	Nutrition bar (South Beach Living High Protein Bar)
	X	X	53720700	Nutrition bar (Tiger's Milk)
	X	X	53720800	Nutrition bar (Zone Perfect Classic Crunch)
	X	X	53729000	Nutrition bar or meal replacement bar, NFS
X			91780010	Snickers Marathon Energy bar
X			91781010	Snickers Marathon Protein bar
			Alcoholic beverages (pre-mixed cocktails, wine coolers, and malt beverages) (low/reduced kcal only)	
			(proposed usage = 4%)	
X	X	X	93102000	Beer, light
	X	X	93102100	Beer, low carb
	X	X	93301183	Whiskey and diet cola
	X	X	93301191	Rum and diet cola
	X	X	93301215	Vodka and diet cola

Daesang: D-psicose (synonymous with D-allulose)

Appendix D. Effects of Calculation Methods on EDI Values

EDIs presented in GRNs 693 and 828 were based on the 2007-2010 NHANES datasets (GRN 828 adopted the EDI values from GRN 693, therefore the database used in GRN 828 should be the 2007-2010 survey – in GRN 828, there was a typo related to the year of NHANES dataset). Thus, EDIs were calculated under the intended use of the current notice using the NHANES 2007-2010 dataset.

Tables D-1 and D-2 present the EDIs based on day 1 NHANES 2007-2010 and D-3 and D4 are based on the averages of the 2-day of the NHANES 2007-2010 NHANES dataset.

As shown in Appendix C, many food codes used in GRN 400 and 623 (NHANES 2007-2010) and GRN 828 (NHANES 2011-2014) have been dropped and are no longer available in the 2017-2018 NHANES dataset, resulting in lower EDIs when using the most recent dietary survey dataset. In addition, using averages of the 2-day data results in lower EDI values.

Table D-1. Maximum Cumulative EDIs of D-Allulose, g/day * (Based on 2-Day averages from the NHANES 2007-2010 Dataset)

Population	N of users	% of users	Per User (g/day)		Per Capita (g/day)	
			Mean	90 th Percentile	Mean	90 th Percentile
2+ yr	12,536	91.0	9.8±0.37	25.3±1.51	8.9±0.34	23.6±1.39
2-5 yr	1,179	93.6	3.5±0.15	8.1±0.43	3.3±0.16	7.9±0.45
6-12 yr	2,029	93.9	4.4±0.16	10.2±0.43	4.2±0.15	9.8±0.38
13-18 yr M+F	1,396	83.4	5.6±0.40	12.1±0.74	4.7±0.34	11.8±0.81
13-18 yr M	682	80.8	5.6±0.46	12.1±0.94	4.6±0.40	11.2±1.07
13-18 yr F	714	86.1	5.5±0.53	12.8±1.14	4.7±0.46	11.8±1.22
19-99 yr M+F	7,932	91.3	11.4±0.43	30.1±1.64	10.4±0.41	27.8±1.53
19-99 yr M	3,750	88.9	12.9±0.61	34.9±2.63	11.5±0.57	32.4±2.57
19-99 yr F	4,182	93.5	9.9±0.44	25.9±1.72	9.3±0.42	25.0±1.71

* Assuming All the Foods will be Used at the Maximum Use Levels.

M=male; F=female; yr=years.

Daesang: D-psicose (synonymous with D-allulose)

Table D-2. Maximum Cumulative EDIs of D-Allulose, g/kg bw/day (Based on 2-day averages of the NHANES 2007-2010 Dataset)

Population	Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
	Mean	90 th Percentile	Mean	90 th Percentile
2+ yr	0.14±0.005	0.34±0.015	0.13±0.004	0.33±0.014
2-5 yr	0.22±0.010	0.49±0.028	0.20±0.010	0.49±0.033
6-12 yr	0.13±0.005	0.30±0.011	0.12±0.005	0.3±0.012
13-18 yr M+F	0.09±0.006	0.22±0.025	0.07±0.005	0.19±0.023
13-18 yr M	0.08±0.006	0.18±0.019	0.07±0.005	0.15±0.013
13-18 yr F	0.09±0.008	0.24±0.031	0.08±0.007	0.23±0.03
19-99 yr M+F	0.14±0.006	0.36±0.017	0.13±0.005	0.33±0.017
19-99 yr M	0.15±0.008	0.39±0.024	0.13±0.007	0.36±0.024
19-99 yr F	0.13±0.006	0.33±0.017	0.12±0.006	0.32±0.014

* Assuming All the Foods will be Used at the Maximum Use Levels.

M=male; F=female; bw=body weight; yr=years.

Table D-3. Maximum Cumulative EDIs of D-Allulose, g/day * (Based on Day 1 NHANES 2007-2010 Dataset)

Population	N of users	% of users	Per User (g/day)		Per Capita (g/day)	
			Mean	90 th Percentile	Mean	90 th Percentile
2+ y	13,688	80.7	12.5±0.30	33.4±1.42	10.1±0.25	28.3±1.03
2-5 y	1,406	83.3	4.1±0.26	9.9±0.99	3.4±0.22	9.0±0.77
6-12 y	2,028	81.3	5.7±0.23	14.2±0.77	4.6±0.21	12.6±0.54
13-18 M+F	1,245	70.4	7.5±0.47	18.4±1.69	5.3±0.38	14.2±1.08
13-18 M	633	66.0	7.8±0.75	21.7±2.23	5.1±0.57	14.7±2.36
13-18 F	612	74.5	7.2±0.58	17.0±2.15	5.4±0.48	14.1±1.38
19-99 M+F	9,009	81.6	14.5±0.38	38.7±1.58	11.8±0.31	34.2±1.48
19-99 M	4,348	79.2	16.8±0.66	48.0±2.30	13.3±0.52	39.7±2.13
19-99 F	4,661	83.9	12.4±0.47	32.5±1.69	10.4±0.41	28.9±1.41

* Assuming All the Foods will be Used at the Maximum Use Levels.

Daesang: D-psicose (synonymous with D-allulose)

Table D-4. Maximum Cumulative EDIs of D-Allulose, g/kg bw/day (Based on Day 1 NHANES 2007-2010)

Population	Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
	Mean	90 th Percentile	Mean	90 th Percentile
U.S. 2+ y	0.18±0.004	0.46±0.015	0.14±0.003	0.4±0.011
2-5 y	0.25±0.015	0.59±0.052	0.21±0.013	0.53±0.041
6-12 y	0.17±0.007	0.41±0.030	0.14±0.007	0.36±0.027
13-18 M+F	0.12±0.007	0.30±0.023	0.09±0.006	0.25±0.019
13-18 M	0.12±0.011	0.30±0.046	0.08±0.008	0.22±0.032
13-18 F	0.12±0.011	0.3±0.029	0.09±0.009	0.25±0.029
19-99 M+F	0.18±0.005	0.48±0.017	0.15±0.004	0.41±0.012
19-99 M	0.19±0.008	0.51±0.021	0.15±0.006	0.46±0.026
19-99 F	0.17±0.006	0.43±0.016	0.14±0.005	0.39±0.015

*Assuming All the Foods will be Used at the Maximum Use Levels.

Daesang: D-psicose (synonymous with D-allulose)

Appendix E. Toxicological Evaluation of Daesang's D-psicose (D-allulose)

This report summarizes the following studies:

- Bacterial reverse mutation study, Biototech, 2022a
- *In vitro* Mammalian chromosomal aberration test, Biototech, 2022b
- *In vivo* mouse micronucleus test, Biototech, 2022c
- Acute toxicity in rats, Biototech, 2022d.

Full reports are available upon request.

1. Objective

The safety of Daesang's D-psicose (D-allulose), produced by the genetically modified *Corynebacterium glutamicum* DS00001 harboring D-psicose epimerase, was evaluated in mutagenicity, two types of genotoxicity studies, and an acute toxicity study in rats.

2. Regulatory Guidelines

The studies were conducted in accordance with Good Laboratory Practice (GLP) regulations for nonclinical laboratory studies from the Ministry of Food, Drug Safety of Korea, Standards for Toxicity Studies of Drugs of Notification from Ministry of Food and Drug Safety of Korea, and Organization for Economic Co-operation and Development (OECD) guidelines for GLP: bacterial reverse mutation study – OECD 471; *in vitro* Mammalian chromosomal aberration test – OECD 473, *in vivo* micronucleus test – OECD 474.

3. Material and Methods

3.1 Test material

D-psicose (D-allulose), white powder, was provided by Daesang Corp. The purity of D-allulose for the studies was 99.9% (dry wt. basis). Daesang's D-allulose is manufactured under cGMP using a common food grade and process.

3.2 Animal Ethics

Biototech Co., Ltd. is a good laboratory practice (GLP)-certified laboratory that has maintained full accreditation from the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) since 2010. The animal studies were conducted under the permission of Institutional Animal Care and Use Committee (IACUC) of Biototech Co, Ltd., based on the Animal Protection Act of the Republic of Korea.

3.3 Test Design

3.3.1 Single Oral Dose Toxicity Study

Daesang: D-psicose (synonymous with D-allulose)

The acute toxicity study was conducted to assess the potential toxicity and to determine the approximate lethal dose of the test substance, D-allulose, following a single oral administration to 8-week-old male and female Sprague-Dawley rats. Test groups consisted of 5 males and 5 females, with each group dosed with 1,250, 2,500, or 5,000 mg/kg. A separate group was injected with water to provide a control. All animals were monitored for clinical signs and body weight changes during the 14-day observation period. At the end of the observation period, all animals were euthanized and subjected to gross necropsy.

3.3.2 Bacterial Reverse Mutation Study

The mutagenicity of the test substance, D-allulose, was evaluated in histidine-requiring *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and a tryptophan-requiring *Escherichia coli* strain [WP2uvrA(pKM101)] in the presence or absence of S9 metabolic activation. In the dose-ranging study, the bacteria were exposed to six doses of D-allulose: 4.88, 19.5, 78.1, 313, 1,250, and 5,000 µg/plate. Growth inhibition and precipitation of D-allulose were not evident at all dose levels. Therefore, the dose levels selected for the main study included: 313, 625, 1,250, 2,500, and 5,000 µg/plate. In addition, negative and positive control groups were set.

Table E-1. Mutagenicity of D-allulose in bacterial culture at 6 dose levels.

Strains	S9 mix	Dose level (µg/plate)
TA98,TA100,TA1535, TA1537, WP2 uvrA	-/+	5,000, 2,500, 1,250, 625, 313

The main study was conducted using the pre-incubation method. All treatments were divided into absence or presence of S9 metabolic activation. Plates were cultured in an incubator at 37°C for 48 hours. The number of revertant colonies was counted by a colony counter or by visual counting.

3.3.3 *in vitro* Mammalian Chromosomal Aberration Test

The ability of the test substance, D-allulose, to induce chromosomal aberrations was evaluated in Chinese Hamster lung (CHL/IU cell line) cells. In the dose range-finding study, the cells were exposed to 20, 39, 78, 156, 313, 625, 1,250, 2,500, and 5,000 µg/mL in the short time treatments with and without metabolic activation, as well as in the continuous treatments without metabolic activation. Metabolic activation included S9 and cofactor C (MgCl₂, KCl, glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate [NADP], and HEPES buffer) at a ratio of 2 to 4.7. Cytotoxicity and precipitation were not evident. Therefore, the following dose levels were selected for the main study: 5,000, 2,500, and 5,000 µg/plate. In addition, negative and positive control groups were set.

Daesang: D-psicose (synonymous with D-allulose)

E2. *in vitro* Mammalian Chromosomal Aberration Test Conditions

Treatment	S9 mix	Dose level ($\mu\text{g}/\text{plate}$)
Short time	-/+	5,000, 2,500, 1,250
Continuous	-	5,000, 2,500, 1,250

The short time treatment of the main study was conducted with or without metabolic activation, and the cells were treated with the test substance for six hours and then cultured for an additional 18 hours in a 5% CO₂ incubator at 37°C. In the continuous treatment without metabolic activation, cells were treated with the test substance for 24 hours in a 5% CO₂ incubator at 37°C.

3.3.4 *In vivo* Micronucleus Test

The ability of D-allulose to induce micronuclei was evaluated in Sprague-Dawley Rat bone marrow cells. Results from a “Single Oral Dose Toxicity Study of Allulose in Sprague-Dawley Rats (Biototech Study No. B22099)”, showed no mortality at the 5,000 mg/kg bw dose level of the test substance in rats. Therefore, 5,000 mg/kg/day was selected as the high dose of this study and two additional lower dose levels (2,500 and 1,250 mg/kg bw/day) were produced by applying a geometric ratio of two. In addition, the positive (cyclophosphamide) and negative (water) control groups were set. Since there was no sex difference in mortality, only males, which are known to be susceptible to induced micronuclei, were used in the main study. The dosing formulation was administered via gastric intubation twice at 24-hour intervals using a disposable syringe fitted with an intubation tube. The positive control substance was administered via gastric intubation once 24 hours prior to harvesting bone marrow cells. All animals were monitored for clinical signs and body weight. They were euthanized with anesthesia after administration of test substance, and bone marrow sample slides of those animals were observed under a microscope.

4. Results and Discussion

4.1 Single Oral Dose Toxicity Study

There were no animal deaths in the 1,250 (low-dose), 2,500 (mid-dose), and 5,000 (high-dose) mg/kg bw groups. No test substance-related effect was observed in body weights and necropsy findings in the 1,250, 2,500, and 5,000 mg/kg bw groups for both sexes. No test substance-related effect was observed in clinical signs in the low-dose group of both sexes. However, diarrhea or mucous stool was observed at 2-6 hours after dosing in both sexes in the mid- and high- dose groups. Based on the results of this study, it is considered the approximate lethal dose of the test substance, D-allulose, is greater than 5,000 mg/kg bw in both male and female rats following a single oral administration under the conditions of this study.

Table E-3. Summary Results of Single Oral Dose Toxicity Study

Sex	Group	Dose (mg/kg)	N	Body weight (g) at Various Days					
				1	2	4	8	15	D 1-15 gain
M	G1	0	5	264.9±4 .2	294.2±4 .8	317.8±6 .6	352.2±9. 1	407.5±1 5.3	142.7± 14.0
	G2	1,250		265.6±9 .5	296.7±8 .9	318.3±1 3.3	350.5±1 4.9	406.4±1 5.0	140.8± 8.5
	G3	2,500		264.1±1 1.2	293.2±5 .1	315.2±8 .2	348.5±1 3.2	402.8±1 7.4	138.7±15 .5
	G4	5,000		262.9±7 .8	294.5±9 .2	313.2±8 .6	347.4±1 1.1	396.6±1 4.1	133.7± 8.4
F	G1	0	5	177.3±2 .8	197.2±4 .1	210.1±7 .2	222.8±1 0.2	242.8±1 5.0	65.5±14. 1
	G2	1,250		177.5±5 .0	194.5±7 .7	208.6±7 .2	219.5±1 1.4	239.9±1 1.2	61.9±7.8
	G3	2,500		176.6±4 .5	196.2±5 .7	208.8±6 .6	221.3±6. 6	243.2±7 .9	66.5±8.5
	G4	5,000		174.6±3 .9	192.1±5 .1	202.5±3 .6	216.6±3. 0	237.9±3 .1	63.6±2.7

There were no significant differences among the groups.

F=Females; M=males.

4.2 Bacterial Reverse Mutation Study

The mean number of revertant colonies was less than twice the negative control group at all dose levels of D-allulose in the absence and presence of metabolic activation. In the positive control group, the mean number of revertant colonies for each strain was markedly increased more than twice when compared to the negative control. Therefore, these results demonstrated that D-allulose did not show any indication of mutagenic potential under the conditions of this study.

Table E-4. Summary Results of Bacterial Reverse Mutation Study

Strain	Test substance	Dose (µg/plate)	Mean revertant colony counts			
			1 st main study		2 nd main study	
			Mean	S.D.*	Mean	S.D.*
TA 98	Water for injection	0	15	2	15	1
	D-psicose (D-allulose)	313	12	1	14	1
		625	12	2	13	1
		1,250	13	2	14	1
		2,500	17	1	16	1
		5,000	16	1	15	1
2-NF*	5.0	573	24	608	13	
TA 100	Water for injection	0	100	3	103	3
	D-psicose (D-allulose)	313	101	4	106	4
		625	107	4	104	2
		1,250	105	3	103	3
		2,500	103	2	106	2

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		5,000	106	4	104	3
	SA*	1.5	563	25	749	16
TA 1535	Water for injection	0	13	1	13	2
	D-psicose (D-allulose)	313	14	2	11	1
		625	10	1	12	1
		1,250	13	2	11	1
		2,500	12	2	13	1
		5,000	15	2	12	3
SA*	1.5	609	14	642	7	
TA 1537	Water for injection	0	9	2	9	1
	D-psicose (D-allulose)	313	7	2	8	1
		625	8	1	9	1
		1,250	7	1	8	1
		2,500	6	2	9	1
		5,000	9	1	9	1
9-A-A*	80.0	610	18	485	11	
WP2 _{uvrA}	Water for injection	0	29	1	31	1
	D-psicose (D-allulose)	313	34	2	33	1
		625	32	2	31	1
		1,250	34	1	35	1
		2,500	32	2	32	1
		5,000	35	2	32	2
4-NQO*	0.3	854	25	599	4	

*S.D.=Standard Deviation; 2-NF=2-Nitrofluorene; SA=Sodium azide; 9-A-A=9-Aminoacridine; 4-NQO=4-Nitroquinoline N-oxide.

4.3 *In vitro* Mammalian Chromosomal Aberration Test

The results of calculations of relative population doubling (RPD) at all dose levels were 93.2% or higher in the short time treatments with and without metabolic activation and in the continuous treatment without metabolic activation. In addition, precipitation of the test substance was not observed. After observation of slides with a microscope, 300 metaphase cells were evident at the high dose in the short time treatments with and without metabolic activation and in the continuous treatment without metabolic activation. Therefore, three dose levels including the high dose were selected for the observation of chromosome aberrations. The frequency of cells with chromosome aberrations in the short time treatments with and without metabolic activation and in the continuous treatment without metabolic activation was not statistically significantly different when compared to the negative control group. Therefore, these results demonstrated that D-allulose did not show any evidence to induce chromosomal aberration under the conditions of this study.

Table E-5. Summary Results of *in vitro* Mammalian Chromosomal Aberration Test

Test substance	Dose (µg/mL)	S9 mix	Trt-Rec Time* (h)	Number of cells with structural aberrations		Number of cells with numerical aberrations
				Total (%)		Total (%)
				gap-	gap+	
Water for injection	0	-	6-18	0(0.0)	0(0.0)	0(0.0)
D-psicose (D-allulose)	1,250	-	6-18	0(0.0)	0(0.0)	0(0.0)
	2,500	-	6-18	0(0.0)	0(0.0)	0(0.0)
	5,000	-	6-18	0(0.0)	0(0.0)	0(0.0)
MMC*	0.1	-	6-18	54**(18.0)	54(18.0)	0(0.0)
Water for injection	0	+	6-18	0(0.0)	0(0.0)	0(0.0)
D-psicose (D-allulose)	1,250	+	6-18	0(0.0)	0(0.0)	0(0.0)
	2,500	+	6-18	0(0.0)	0(0.0)	0(0.0)
	5,000	+	6-18	1(0.3)	2(0.7)	0(0.0)
B[a]P*	20	+	6-18	53**(17.7)	53(17.7)	0(0.0)
Water for injection	0	-	24-0	0(0.0)	0(0.0)	0(0.0)
D-psicose (D-allulose)	1,250	-	24-0	0(0.0)	0(0.0)	0(0.0)
	2,500	-	24-0	0(0.0)	0(0.0)	0(0.0)
	5,000	-	24-0	0(0.0)	0(0.0)	0(0.0)
MMC*	0.1	-	24-0	62**(20.7)	62(20.7)	0(0.0)

Number of cells analyzed = 150 cells.

*MMC=Mitomycin C; B[a]P=Benzo[a]pyrene; Trt-Rec Time=treatment-recovery times;

Gap-=total number of cells with structural aberrations excluding gap; gap+=total number of cells with structural aberrations including gap.

**Significant difference from negative control by Fisher's exact test: p<0.01.

4.4 *in vivo* Micronucleus Test

The results of this study indicate that the incidence of micronucleated polychromatic erythrocytes (MNPCE) in polychromatic erythrocytes (PCE) in the test substance groups was not statistically significantly different from the negative control group. In addition, the ratio of PCE to total erythrocytes in the test substance groups was not significantly different from the negative control group. In the positive control group, the incidence of MNPCE in PCE increased significantly compared to the negative control group. The ratio of PCE to total erythrocytes in the positive control group was not significantly different from the negative control group. Therefore, these results demonstrate that D-allulose did not have any potential to induce micronuclei formation in rat bone marrow cells under the conditions of the study.

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Table E-6. Summary Results of *in vivo* Micronucleus Test in Male Sprague-Dawley Rats

Groups		Dose (mg/kg /day)	Route	Hours After dosing	PCE/ (PCE+NCE) (%)	MNPCE/ PCE (%)
Negative control	Water for injection	0	P.O.*	24	31.7±0.66	0.040±0.014
Test substance	D-psicose (D-allulose)	1,250	P.O.*	24	31.6±0.52	0.045±0.021
		2,500	P.O.*	24	31.8±0.74	0.035±0.014
		5,000	P.O.*	24	31.7±0.81	0.045±0.011
Positive control	CPA*	20	P.O.*	24	31.5±0.59	1.190±0.101

*P.O.=Per Os; CPA=Cyclophosphamide; *MNPCE=Micronucleated polychromatic erythrocyte; NCE=Normochromatic erythrocyte; PCE=Polychromatic erythrocyte.

There were no significant differences among the groups.

5. References

- Biototech, 2020a. Bacterial reverse mutation test of allulose
- Biototech, 2020b. *In vitro* mammalian chromosome aberration study of allulose
- Biototech, 2020c. *In vivo* micronucleus test of allulose
- Biototech, 2022d. Single oral dose toxicity study of allulose

Full reports are available upon request.

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Appendix F. Expert Panel Report

D-psicose (D-allulose) is a monosaccharide that is produced by bioconversion of fructose syrup via the genetically modified *Corynebacterium glutamicum* possessing D-psicose-3-epimerase activity. *Corynebacterium glutamicum* has been safely used as a production microorganism to produce various food ingredients. No production microorganism is present in the finished D-psicose ingredients that are intended to be added to foods. Daesang's D-psicose exists in two forms: crystalline and syrup. The final D-psicose ingredients, crystalline D-psicose and D-psicose syrups, contain $\geq 98\%$ and $\geq 95\%$ D-psicose (dry weight basis; syrup, $\geq 65\%$ on an as-is basis), respectively, and meet appropriate food-grade specifications.

D-psicose is intended for use at levels providing up to 2 to 100 g per 100 g food in selected foods as listed in Table 1.

Table F-1. Intended Use and Maximum Use Levels of D-psicose (D-allulose), % (w/w)

Group No	Description	Maximum use levels, % (w/w)
1	Bakery products (rolls, cakes, pies, pastries, and cookies: low calorie or dietetic)	10
2	Beverages, carbonated: low calorie	3.5
3	Beverages Non-carbonated (non-alcoholic) low calorie, reduced calorie, sugar-free)	3.5
4	Cereals, ready to eat (<5% sugar)	10
5	Cereals: low-and reduced calorie, sugar free	5
6	Cereals, regular	2
7	Chewing gum	50
8	Coffee mix	30
9	Confections and frostings	5
10	Dressings for salads	5
11	Fat-based cream - used in modified fat/calorie cookies, cakes, pastries, and pie	10
12	Frozen dairy desserts (ice cream, soft serve, sorbet: low calorie, reduced calorie, sugar free)	5
13	Gelatins, pudding, fillings (low calorie, reduced calorie, sugar free)	10
14	Hard candies (low calorie)	70
15	Soft candies (non-chocolate, plain chocolate, chocolate coated: low calorie, reduced calorie, sugar free)	25
16	Jams and jellies	10
17	Sugar	10
18	Sugar substitutes	100
19	Sweet sauces and syrups (low calorie, reduced calorie, and sugar free)	10

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20	Yogurt (regular and frozen: low calorie, reduced calorie, sugar free)	5
21	Nutrition bar, grain-based cereal bars, protein bars	15
22	Alcoholic beverages (premixed cocktails, wine cooler, hard seltzer, and malt beverage: low- or reduced calorie; low-or reduced sugar)	3.5

w/w=weight/weight.

The mean and 90th percentile all-user intakes of D-psicose were determined to be 9.1 and 21.3 g/person/day, respectively, under the intended use when the Day 1 2017-2018 NHANES dataset was used for calculation of estimated daily intakes (EDIs). Corresponding EDIs are 7.5 and 17.2 g/person/day, respectively, based on 2-day averages of the 2017-2018 NHANES dataset. The 90th percentile EDI values on a body weight basis did not exceed 0.5 mg/kg bw in any age or gender groups when averages of 2-day survey datasets were used. When Day 1 survey data only were used, the 90th percentile EDI values did not exceed 0.6 mg/kg bw in any age or gender groups. These EDIs are within the safe intake levels found from animal and human clinical studies. In addition, these EDI values are highly amplified because it is not likely that D-psicose will be used at the maximum use levels in all food categories under the intended use and short-term dietary surveys tend to overestimate EDIs (Lambe and Kearney, 1999).

In the previous GRAS notices (GRNs 400, 498, 693, 828, and 1024; FDA, 2012, 2014, 2017, 2020, 2023) to the FDA, the safety of D-allulose has been established in animal toxicity and mutagenicity studies, and is further supported by human clinical studies. The specification and composition of D-psicose preparations are substantially equivalent among various preparations. Data from toxicity studies of Daesang's D-allulose and other sources of D-psicose support the safety of Daesang's D-psicose. The safety of D-psicose is supported by a series of toxicity studies, such as mutagenicity (Huntington Lab, 2011, cited in GRN 400), two types of genotoxicity (Huntington Lab, 2011, cited in GRN 400), acute toxicity (Matsuo et al., 2002), 90-day subchronic toxicity (An et al., 2019), reproductive toxicity (Kim et al., 2019), teratogenicity (Sa et al., 2022), and chronic toxicity studies (Yaji and Matsuo, 2009). From a 90-day oral subchronic toxicity study in rats, the no-observed-adverse effect-level (NOAEL) of D-psicose was determined to be 5,000 mg/kg bw/day, the highest level tested (An et al., 2019). The NOAEL found from a 12- to 18- month chronic toxicity study of another source of D-psicose was 3% of diet or 1,280 mg/kg bw/day, the highest level tested (Yaji and Matsuo, 2009).

Daesang's production microorganism was derived from *Corynebacterium glutamicum* ATCC 13032, which is widely used in the production of various food ingredients, including 2-fucosyllactose (GRN 932, FDA, 2021). The *Corynebacterium glutamicum* species is regarded as having a qualified presumption of safety (QPS) when used as a production organism for amino acids and other food or feed ingredients (EFSA, 2021). D-psicose-3-epimerase is composed of 294 amino acids, and its amino acid sequence did not show any homology with those of known allergenic and toxicogenic proteins when they were screened using

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<http://allergenonline.org/databasefasta.shtml> and the Basic Local Alignment Search Tool for Protein (BLAST) version 2.7.0 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>; accessed on September 6, 2017), respectively. Total protein content is less than 0.02%. It is not likely that D-psicose causes allergenic reactions.

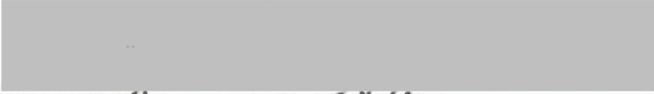
Human tolerance studies of D-psicose showed that single doses up to 0.4 g/kg bw or escalating daily doses up to 0.9 g/kg bw/day were well-tolerated in adults (Han et al., 2018a). Human clinical studies in which D-psicose was administered at doses up to 15 g/day for periods ranging from 12 to 48 weeks indicate that consumption of D-psicose does not result in any adverse effects on measured outcomes, such as glucose and lipid metabolism indicators, as well as liver and kidney function indicators (Han et al., 2018b; Hayashi et al., 2010; Tanaka et al., 2020, 2021). Overall, the safety of D-psicose under the intended use has been demonstrated though the available data from toxicological and clinical studies conducted to date.

We conclude that the intended use of D-psicose (D-allulose) is GRAS based on scientific evidence, and that other experts qualified to assess the safety of foods and food ingredients would concur with these conclusions. Therefore, not only is the proposed use of D-allulose safe within the terms of the Federal Food, Drug, and Cosmetic Act (meeting the standard of reasonable certainty of no harm), but because of this consensus among experts, it is also GRAS according to Title 21 Code of Federal Regulations (21 CFR).

Signatures are on the following page.

Daesang: D-psicose (synonymous with D-allulose)

Expert Panel Members



George C. Fahey, Jr., Ph.D.
Professor Emeritus, University of Illinois, Urbana, IL

4/18/23
Date



Joanne Slavin, Ph.D., R.D.
Professor, University of Minnesota, St. Paul, MN

4-13-2023
Date



Yong-su Jin, Ph.D.
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4/21/23
Date

Advisor to the Expert Panel



Susan Cho, Ph.D.
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4/23/23
Date

Daesang: D-psicose (synonymous with D-allulose)

REFERENCES for APPENDIX F

- An M, Lee J, Park YC, Park C, Kim HJ. 90-Day repeated oral toxicity test of D-psicose produced from *Microbacterium foliorum*. Regul Toxicol Pharmacol. 2019;109:104485.
- European Food Safety Authority (EFSA). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 13: Suitability of taxonomic units notified to EFSA until September 2020. EFSA J. 2021;19(1):6377.
- Food and Drug Administration (FDA). 2012. GRN 400, D-psicose, filed by CJ Cheiljedang, South Korea. Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=400>.
- FDA. 2014. GRN 498, D-psicose, filed by Matsutani Chemical Industry, Japan. Available at: <http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=498>.
- FDA. 2017. GRN 693, D-psicose, filed by Samyang Corp., South Korea. Available at: GRAS Notice 693, D-psicose (fda.gov).
- FDA. 2020. GRN 828, D-psicose, filed by Samyang Corp., South Korea. Available at: GRAS Notice 828, D-psicose (fda.gov).
- FDA. 2021. GRN 932, 2-fusocyllactose, filed by Advanced Protein Technologies Corp., South Korea. Available at: GRAS Notice 932, Agency Response Letter (fda.gov).
- FDA. 2023. GRN 1024, D-psicose, filed by Blue California, USA. Available at: GRAS Notice 1024, D-psicose (fda.gov).
- Han Y, Choi BR, Kim SY, Kim SB, Kim YH, Kwon EY, Choi MS. Gastrointestinal tolerance of D-allulose in healthy and young adults. A non-randomized controlled trial. Nutrients. 2018a;10:2010.
- Han Y, Kwon EY, Yu MK, Lee SJ, Kim HJ, Kim SB, Kim YH, Choi MS. A preliminary study for evaluating the dose-dependent effect of D-allulose for fat mass reduction in adult humans: A randomized, double-blind, placebo-controlled trial. Nutrients. 2018b;10. pii: E160.
- Hayashi N, Iida T, Yamada T, Okuma K, Takehara I, Yamamoto T, Yamada K, Tokuda M. Study on the postprandial blood glucose suppression effect of D-psicose in borderline diabetes and the safety of long-term ingestion by normal human subjects. Biosci Biotechnol Biochem. 2010;74:510-19.
- Huntington Lab. 2011. GRN 400, US FDA, 2011. Original reports or details of the studies are not available.
- Kim H, Park C, Sa S, Case I, Li C, Gao Y, Wang H, Tian J. A study of D-allulose-associated reproductive toxicity in rats. Food Chem Toxicol. 2019;131:110548.

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Lambe J, Kearney J. The influence of survey duration on estimates of food intakes: Relevance for food-based dietary guidelines. *Br J Nutr.* 1999;81 Suppl 2:S139-42.

Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Effects of oral acute administration and subchronic feeding of several levels of D-psicose in rats. *J Nutr Sci Vitaminol.* 2002;48:512-16.

Sa S, Seol Y, Lee AW, Heo Y, Kim HJ, Park CJ. Teratogenicity of D-allulose. *Toxicol Rep.* 2022;9:821-24.

Tanaka M, Kanasaki A, Hayashi N, Iida T, Murao K. Safety and efficacy of a 48-week long-term ingestion of D-allulose in subjects with high LDL cholesterol levels. *Fundamental Toxicol Sci.* 2020;7:1-15.

Tanaka M, Kanasaki A, Hayashi N, Iida T, Murao K. The influence of long-term ingestion of D-allulose in hypercholesterolemia patients under statin therapy. *Fundamental Toxicol Sci.* 2021;8:23-31

Yagi K, Matsuo T. The study on long-term toxicity of D-psicose in rats. *J Clin Biochem Nutr.* 2009;45:271-77.

FDA USE ONLY

GRN NUMBER 001148	DATE OF RECEIPT
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

**GENERALLY RECOGNIZED AS SAFE
(GRAS) NOTICE (Subpart E of Part 170)**

Transmit completed form and attachments electronically via the Electronic Submission Gateway (see Instructions); OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety (HFS-200), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission (Check one)

New Amendment to GRN No. _____ Supplement to GRN No. _____

2. All electronic files included in this submission have been checked and found to be virus free. (Check box to verify)

3. Most recent presubmission meeting (if any) with FDA on the subject substance (yyyy/mm/dd): _____

4. For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? (Check one)
 Yes If yes, enter the date of communication (yyyy/mm/dd): _____
 No

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier	Name of Contact Person Jason Park	Position or Title Director	
	Organization (if applicable) Daesang Corporation		
	Mailing Address (number and street) 120, Changgyeonggung-ro, Jongno-gu		
City Seoul	State or Province Seoul	Zip Code/Postal Code 03130	Country Korea, Republic of
Telephone Number +82-2-490-5068	Fax Number	E-Mail Address sh_0421@daesang.com	
1b. Agent or Attorney (if applicable)	Name of Contact Person Susan Cho	Position or Title Chief Science Officer	
	Organization (if applicable) AceOne RS, Inc.		
	Mailing Address (number and street) 5903 Hampton Forest Way		
City Fairfax	State or Province Virginia	Zip Code/Postal Code 22030	Country United States of America
Telephone Number 301-875-6454	Fax Number	E-Mail Address scho@aceoners.com	

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

D-psicose (synonymous with D-allulose)

2. Submission Format: *(Check appropriate box(es))*

- Electronic Submission Gateway Electronic files on physical media
 Paper
If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

- Yes *(Proceed to Item 5)* No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

- a) GRAS Notice No. GRN 000693
 b) GRAS Affirmation Petition No. GRP _____
 c) Food Additive Petition No. FAP _____
 d) Food Master File No. FMF _____
 e) Other or Additional *(describe or enter information as above)* _____

6. Statutory basis for conclusions of GRAS status *(Check one)*

- Scientific procedures *(21 CFR 170.30(a) and (b))* Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? *(see 21 CFR 170.225(c)(8))*

- Yes *(Proceed to Item 8)*
 No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

- Yes, information is designated at the place where it occurs in the submission
 No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

- Yes, a redacted copy of the complete submission
 Yes, a redacted copy of part(s) of the submission
 No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Daesang proposes to use D-psicose (D-allulose) as a food ingredient in selected foods at use levels of 2-100 g/100 g: bakery products (rolls, cakes, pies, pastries, and cookies: low calorie or dietetic); low-calorie beverages; selected cereals; chewing gums; coffee mix; confections and frostings; dressings for salads; fat-based creams; frozen dairy desserts (ice cream, soft serve, and sorbet: low-calorie, reduced-calorie, and sugar-free); gelatins, pudding, and fillings (low-calorie, reduced-calorie, and sugar-free); hard candies (low-calorie); soft candies (non-chocolate, plain chocolate, and chocolate coated; low-calorie, reduced-calorie, and sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low-calorie, reduced-calorie, and sugar-free); yogurt (regular and frozen: +

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

- Yes No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

- Yes No, you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

- PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).
- PART 3 of a GRAS notice: Dietary exposure (170.235).
- PART 4 of a GRAS notice: Self-limiting levels of use (170.240).
- PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).
- PART 6 of a GRAS notice: Narrative (170.250).
- PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

Yes No

Did you include this other information in the list of attachments?

Yes No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Daesang
(name of notifier)
has concluded that the intended use(s) of D-psicose (synonymous with D-allulose)
(name of notified substance)
described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Daesang Corporation
(name of notifier) agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

Jongno Place, 120, Changgyeonggung-ro, Jongno-gu, Seoul, Republic of Korea
(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance. The notifying party certifies that the information provided herein is accurate and complete to the best of his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official,
Agent, or Attorney

Printed Name and Title

Jason Park, Director

Date (mm/dd/yyyy)

05/04/2023

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	D-psicoseGRAScoverletter-Daesang5-4-23.pdf	Administrative
	D-psicoseGRASbyDaesang5-4-23FinalsubmittedtoFDA.pdf	Administrative

OMB Statement: Public reporting burden for this collection of information is estimated to average 170 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to: Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, PRASStaff@fda.hhs.gov. (Please do NOT return the form to this address.). An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

Hice, Stephanie

From: Susan S Cho <susanscho1@yahoo.com>
Sent: Monday, March 18, 2024 2:11 PM
To: Hice, Stephanie
Subject: [EXTERNAL] Amendment to GRN 001148 D-psicose (D-allulose)
Attachments: GRN001148 Amendment 3-18-24 submitted to FDA r.pdf

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear Dr. Hice,

Good afternoon! Please see an amendment to GRN 001148 after corrections for 2 typos on p20. I apologize for the inconvenience. Have a nice day!

Sincerely,
Susan
Susan Cho, Ph.D.
AceOne RS, Inc. (formerly NutraSource, Inc.)
Agent for Daesang +1-301-875-6454 (C)



March 18, 2024

To: Dr. Stephanie Hice, FDA

Subject: Amendment to GRN 001148, D-psicose (or D-allulose)

From: Susan Cho, AceOne RS, Inc., Agent for Daesang

Dear Dr. Hice,

In response to FDA's questions, Daesang has prepared its amendment as follows:

1. Please provide the strain name for the *Corynebacterium glutamicum* production strain, and please state whether the production strain has been deposited in a recognized culture collection and provide the corresponding deposit designation. If it has not been deposited, please discuss how the strain was taxonomically identified and verified.

Daesang's response

Daesang's production strain is *Corynebacterium glutamicum* DS00001, which is deposited as KCCM 80101 at Korea Bank of Strains, Korean Culture Collection of Microorganisms (KCCM).

2. On page 58, the notifier states "Daesang's production microorganism was derived from *Corynebacterium glutamicum* ATCC 13032, which was obtained from the U.S. Bank of Strains". Please clarify what repository the "U.S. Bank of Strains" refers to.

Daesang's response

Daesang's production strain was derived from *Corynebacterium glutamicum* ATCC 13032, which is deposited at American Type Culture Collection (ATCC). We apologize for the mistranslation, 'which was obtained from the U.S. Bank of Strains'.

3. Please provide a detailed description of the *C. glutamicum* production strain including genotypic (e.g., pathogenicity and toxigenicity) and phenotypic characteristics (e.g., production of antimicrobials, production of secondary metabolites and antimicrobial resistance), and whether this poses a safety concern.

Daesang's response

The genetically modified strain, DS00001, is deposited as KCCM 80101 at Korean Culture Collection of Microorganisms. It is originated from *C. glutamicum* ATCC13032. The genome sequence of the DS00001 (also KCCM 80101) strain comprises 2 contigs with a combined length of 3,288,137 bp, the C+G content of 53.8% and more than 3,000 annotated protein coding sequences (CDS) (Appendix A). The genome sequence of parent strain ATCC13032 is very similar to that of DS00001: it comprises a circular chromosome with a length of 3,282,708 bp, the C+G content of 53.8% and more than 3,000 CDS (Table 5 of Appendix A of this amendment).

Table 1. Description of the test strain genome and comparison to *Corynebacterium glutamicum*.

Description of the test strain genome	Test strain	Data for <i>Corynebacterium glutamicum</i> from NCBI GenBank	Data for <i>Corynebacterium glutamicum</i> ATCC 13032 parent strain
Search date	NA	07/12/2022	07/12/2022
Available genomes	NA	75	1
Number of contigs	2	NA	1
Total size of the annotated genome	3,288,137 bp	3,265,960 bp	3,282,708 bp
% C/G	53.8	54.1	53.8
Genes	3,128	NA	3,046
CDSs	3,028	2,854	2,966
rRNAs	18	NA	18
tRNAs	60	NA	59
tmRNA	1	NA	1
Miscellaneous RNAs	21	NA	NA
Plasmids	1	NA	None

From BioSafe Ltd. Study Report: Bioinformatic analysis of *Corynebacterium glutamicum* KCCM 80101 based on whole genome sequencing data; Table 5.

Abbreviations: CDSs = coding sequences; C/G = cytosine/guanine; NA = not available; NCBI = National Center for Biotechnology Information; RNA = ribonucleic acid; rRNA = ribosomal RNA; tRNA = transfer RNA; tmRNA = transfer-messenger RNA.

Phenotypic characteristics

Corynebacterium glutamicum is a gram-positive and non-pathogenic bacterium that occurs naturally in soil and is recognized as safe (Kang et al., 2020). *Corynebacterium* is commonly found in soil, water, plants, and food. Several species of *Corynebacterium* are also found in the skin mucosa of humans and animals (Collins et al., 2004; Yassin, 2003). The mode of propagation of organisms is the same as that of ordinary microorganisms, which proliferate during cell division and do not form spores (Yassin, 2003).

No literature reports pathogenicity and toxigenicity of *C. glutamicum*. Thus, *C. glutamicum* has established a European Food Safety Authority (EFSA) qualified presumption of safety (QPS) status for production purpose (EFSA, 2007, 2024).

Genotypic characteristics

Two potential antimicrobial resistance genes exceeding the identity and coverage thresholds were identified in the genome of the *C. glutamicum* KCCM 80101 (DS00001). Gene *cmr*, which encodes a multi-drug efflux MFS transporter protein of *Corynebacterium glutamicum* and is intrinsic and not associated with mobile genetic elements, was identified. Also, the gene *nptII*, which was included as a part of the genetic modification, was identified. *NptII* encodes aminoglycoside 3'-phosphotransferase and inactivates aminoglycoside antibiotics like kanamycin and is used widely as a selectable marker.

MIC values (Appendix B of This Amendment)

As shown in Table 2, *C. glutamicum* ATCC 13032, the parent strain of KCCM 80101 (DS00001), was susceptible to various antibiotics. The MIC-values for ampicillin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol, vancomycin, and kanamycin against *C. glutamicum* ATCC 13032 were equal or below the EFSA MIC cut-off values.

In the case of *C. glutamicum* KCCM 80101 (DS00001), the MIC-values for all antibiotics tested were equal or below the EFSA MIC cut-off values with the exception of kanamycin: the MIC value for kanamycin exceeded eight-fold the EFSA cut-off values for *Corynebacterium* (Biosafe report T1493R1502/2022). It is due to the kanamycin selection biomarker (*nptII*) being introduced with the genetic modifications to enable the production of allulose. However, *C. glutamicum* KCCM 80101 (DS00001) was not capable of transferring the *nptII* gene to co-cultured microorganisms. Details are described in Appendix C of this amendment.

Table 2. Susceptibility of *C. glutamicum* ATCC 13032 and *C. glutamicum* DS00001 (or KCCM 80101) to the antimicrobials required to be tested by EFSA.

Antimicrobial	<i>C. glutamicum</i> ATCC 13032 MIC (µg/mL)*	<i>C. glutamicum</i> DS00001 MIC (µg/mL)**	EFSA MIC cut-off values for <i>Corynebacterium</i> (µg/mL)
Ampicillin	0.25	≤0.25 ^a	1
Gentamicin	≤0.03	≤0.12	4
Kanamycin	0.06	128^b	16
Streptomycin	0.5	≤2	8
Tetracycline	0.5	0.5 ^c	2
Erythromycin	0.25	0.5	1

Clindamycin	2	2	4
Chloramphenicol	2	4	4
Vancomycin	0.5	≤0.25	4

“From Table 2 of the Culture Collection of Antimicrobial Resistant Microbes (ARM) Study Report (Appendix B): Determination of the minimum inhibitory concentration of selected antimicrobials against *Corynebacterium glutamicum* spp.

** From Table 2 of Biosafe Ltd. Study Report: Determination of the minimum inhibitory concentration of selected antimicrobials against *Corynebacterium glutamicum* KCCM 80101 (Appendix C).

The values are mode of three replicate analyses (values that differ from the mode are noted in the footnote).

^a The result for ampicillin was recorded after 48h.

^b One of the three replicates presented a MIC of 256 µg/mL.

^c One of the three replicates presented a MIC of 1 µg/mL.

Abbreviations: EFSA = European Food Safety Authority; MIC = minimum inhibitory concentration.

References

Kang KY, Kim MS, Lee MS, Oh JJ, An S, Park D, Heo IK, Lee HK, Song SW, Kim SD., 2020. Genotoxicity and acute toxicity evaluation of the three amino acid additives with *Corynebacterium glutamicum* biomass. *Toxicol Rep.* 7:241-253.

Collins, M. D., Hoyles, L., Foster, G., and Falsen, E. (2004). *Corynebacterium caspium* sp. nov., from a Caspian seal (*Phoca caspica*). *Int. J. Syst. Evol. Microbiol.* 54, 925-928

EFSA Scientific Committee, 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. *EFSA Journal* 2007;5(12):587, 16 pp. <https://doi.org/10.2903/j.efsa.2007.587>.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2024. Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 19: Suitability of taxonomic units notified to EFSA until September 2023. *EFSA J.* 2024 ;22:e8517.

Yassin, A. F., Kroppenstedt, R. M., and Ludwig, W. (2003). *Corynebacterium glaucum* sp. nov. *Int. J. Syst. Evol. Microbiol.* 53, 705-70

4. The notifier describes the *C. glutamicum* production strain as “... harboring *D psicose* 3-epimerase from *Flavonifractor plautii*” (page 14). Please briefly discuss the genotypic and phenotypic characteristics (e.g., pathogenicity, toxigenicity) of *F. plautii* and whether this poses a safety concern.

Daesang's response

Flavonifractor plautii (formerly *Eubacterium plautii*) is highly prevalent in the human gastrointestinal tract, and is known to be a proficient producer of butyrate (Rajilić-Stojanović and DeVos, 2014). It is a gram-positive, strictly anaerobic, and has previously been known as *Eubacterium plautii* or *Clostridium orbiscindis* (Carlier et al. 2010). Cells are described as straight or slightly curved rods, 2-10 microns long, and occur singly or in pairs (Carlier et al. 2010). *Flavonifractor plautii* has a genome consisting of a single circular chromosome with 3,985,392 base pairs, with a G+C content of 60.9% (Tourlousse et al., 2020). The genome is predicted to contain 3 complete sets of rRNA genes, 63 tRNA genes, and 3,764 protein-coding sequences. The American Type Culture Collection (ATCC) considers *F. plautii* to be a Biosafety Level 1 (BSL1) organism, indicative of a low-risk microorganism that poses little to no risk of infection in healthy adults. The Biosafety Level established by Center for Disease Control and Prevention (CDC) is to assess the risks associated with the laboratory activities that occur either from the samples or the procedural requirements, and thus, it may not be relevant when evaluating the safety of microorganisms in the food industry. However, Biosafety Level 1 may indicate a less hazardous microorganism compared to that belonging to Biosafety Level 2-4.

Phenotypic Characteristics

Flavonifractor plautii is capable of metabolizing or degrading plant-derived flavones and flavonoids (Yang et al., 2021). The key enzyme of the metabolic pathway has been identified as a flavone reductase that specifically catalyzes the hydrogenation of the C2–C3 double bond of flavones/flavonols (Yang et al., 2021).

Although a few cases of opportunistic infections have been reported (Berger et al, 2018; Karpat et al., 2021; Strachinaru et al., 2022; Wilton et al., 2022), they are mostly associated with severe predisposing factors, such as following acute cholecystitis (Berger et al., 2018), infectious colitis (Karpat et al., 2021), severe burning (Strachinaru et al., 2022), and hip replacement (Wilton et al., 2022), etc. Due to the fact that *F. plautii* is a common member of the human gut microbiome, it is not expected that *F. plautii* is toxigenic or pathogenic in healthy adults.

Opportunistic infections are not uncommon for many common bacteria including *Lactobacilli*, in particular Generally Recognized As Safe (GRAS) strains such as *Lactobacillus rhamnosus* GG strain (FDA, 2007, GRN 000231 for infant formula application, filed by Mead Johnson), which has a long history of safe use. Salminen et al. (2004) summarizes the bacteremia associated with *Lactobacillus* species as follows: ‘We have reviewed the risk factors and outcome for 89 case patients with *Lactobacillus* bacteremia. Species characterization was done in 53% of the cases, revealing 25 *L. rhamnosus* strains and 22 other *Lactobacillus* species. In 11 cases, the strain was

identical with the probiotic *L. rhamnosus* GG. In 82% of the cases, the patients had severe or fatal comorbidities.’

In summary, there is no evidence that *Flavonifractor plautii* is pathogenic or toxigenic in healthy adults.

Genotypic Characteristics

Berger et al. (2018) conducted the antibiotic resistance test using a gradient test (E-Test, Liofilchem, Roseto degli Abruzzi, Italy) on Columbia agar under anaerobic conditions after 48 h of incubation. The interpretation of minimal inhibitory concentrations (MICs) was carried out in accordance with the European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria (version 8.0, 2018). As shown in Table 3, *F. plautii* was susceptible to a large variety of substances including ampicillin, cefuroxime, ceftriaxone, ceftriaxone, piperacillin/tazobactam, meropenem, imipenem, ertapenem, moxifloxacin, vancomycin, Metronidazole, clindamycin, tigecyclin, and chloramphenicol. However, resistance was found to levofloxacin and ciprofloxacin with intermediate resistance to penicillin and linezolid with low or intermediate MIC values: all the MIC values ranged from 0.5 to 32 mg/L.

In summary, *F. plautii* was susceptible to various antibiotics of clinical significance.

Table 3. Antimicrobial resistance patterns of *Flavonifractor plautii*, minimal inhibitory concentration (MIC).

Substance	MIC (mg/L)	EUCAST (breakpoint range in mg/L)
Penicillin	0.5	intermediate (0.25-0.5) ^a
Ampicillin	0.5	sensitive (4-8) ^a
Cefuroxime	4.0	sensitive (4-8) ^b
Ceftriaxone	0.75	sensitive (1-2) ^b
Piperacillin/tazobactam	0.19	sensitive (8-16) ^a
Meropenem	0.023	sensitive (2-8) ^a
Imipenem	0.064	sensitive (2-8) ^a
Ertapenem	0.023	sensitive (1) ^a
Gentamicin	4.0	-- ^c
Cotrimoxazole (trimethoprim-sulfamethoxazole)	32	-- ^c
Ciprofloxacin	32	resistant (0.25-0.5) ^b
Levofloxacin	2.0	resistant (0.5-1) ^b
Moxifloxacin	0.5	sensitive (0.25) ^b
Vancomycin	2.0	sensitive (2) ^a
Teicoplanin	0.25	-- ^c
Daptomycin	8.0	-- ^c
Linezolid	3.0	intermediate (2-4) ^b
Metronidazole	0.25	sensitive (4) ^a

Clindamycin	0.38	sensitive (4) ^a
Tetracyclin	0.094	-- ^c
Tigecyclin	0.0160	sensitive (0.25-0.5) ^b
Chloramphenicol	0.125	sensitive (8) ^a
Rifampicin	3.0	-- ^c

Breakpoints according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) for gram-positive anaerobes and non-species related breakpoints; for sensitive values the interpretation is \leq for resistant ones $>$ (version 8.0 2018).^a EUCAST breakpoints for gram positive anaerobes utilized.

^b EUCAST non-species related breakpoints utilized.

^c No breakpoints available.

Other considerations

Mixed associations between *F. plautii* and health parameters were reported in the literature. Positive effects: administration of *F. plautii* improved recovery from acute colitis in mice and *F. plautii* can generate desaminotyrosine (from the flavone apigenin) which may protect the human host from influenza (Steed et al., 2017). Negative association: observational studies reported positive associations between the abundance of fecal *F. plautii* and increased risks of colon cancer, enterocolitis, or lung adenocarcinoma (Arnaud et al., 2022; Gupta et al., 2019; Guo et al., 2023). In addition, observational studies do not imply a cause-effect relationship. Although Gupta et al. (2019) hypothesized that extensive degradation of flavonoids by gut microflora may result in lower overall bioavailability of intact flavonoids to minimize the potential beneficial effects and that the degradation products may be contributing to the cancer, there is no indication that *F. plautii* itself poses such health concerns.

In summary, there is no indication that *F. plautii* is pathogenic or toxigenic in healthy individuals.

Additionally, it is noteworthy that Daesang synthesizes the gene encoding for D-psicose-3-epimerase *de novo* with no potential carryover of the donor organism. Only well-characterized DNA fragments, limited solely to the gene encoding for D-psicose-3-epimerase from the donor strain as well as 2 promoters (*sod* and *nptII*) and a replication site, are used in the construction of the genetically modified production strain. The introduced DNA does not encode for any known harmful or toxic substances, as evidenced by amino acid sequence of the enzyme and no homology with known allergenic or toxigenic proteins. If there are any safety concerns, it may be related to the production microorganism instead of a donor microorganism. The safety of Daesang's production microorganism is proven and there is no residual production microorganism in the finished D-psicose ingredient (please see Daesang's answer to FDA's question 3).

In summary, there are no concerns related to the safety of *F. plautii* as a donor organism for Daesang's D-psicose-3-epimerase expressed in *C. glutamicum* KCCM 80101 (DS00001).

References

- Arnaud AP, Cousin I, Schmitt F, Petit T, Parmentier B, Levard G, Podevin G, Guinot A, DeNapoli S, Hervieux E, Flaum V, De Vries P, Randuineau G, David-Le Gall S, Buffet-Bataillon S, Boudry G. Different Fecal Microbiota in Hirschsprung's Patients With and Without Associated Enterocolitis. *Front Microbiol.* 2022;13:904758.
- Berger FK, Schwab N, Glanemann M, Bohle RM, Gärtner B, Groesdonk HV. *Flavonifractor (Eubacterium) plautii* bloodstream infection following acute cholecystitis. *IDCases.* 2018;14:e00461.
- Carlier JP, Bedora-Faure M, K'ouas G, Alauzet C, Mory F. Proposal to unify *Clostridium orbiscindens* Winter et al. 1991 and *Eubacterium plautii* (Séguin 1928) Hofstad and Aasjord 1982, with description of *Flavonifractor plautii* gen. nov., comb. nov., and reassignment of *Bacteroides capillosus* to *Pseudoflavonifractor capillosus* gen. nov., comb. nov. *Int J Syst Evol Microbiol.* 2010;60(Pt 3):585-590.
- FDA 2007, GRN000231. *Lactobacillus casei* subsp. *rhannosus* strain GG, filed by Mead Johnson. Available at: GRAS Notice Inventory > Agency Response Letter GRAS Notice No. GRN 000231 ([archive-it.org](https://www.fda.gov/oc/gras-notice-inventory)).
- Guo Y, Yuan W, Lyu N, Pan Y, Cao X, Wang Y, Han Y, Zhu B. Association Studies on Gut and Lung Microbiomes in Patients with Lung Adenocarcinoma. *Microorganisms.* 2023;11(3):546.
- Gupta A, Dhakan DB, Maji A, Saxena R, P K VP, Mahajan S, Pulikkan J, Kurian J, Gomez AM, Scaria J, Amato KR, Sharma AK, Sharma VK. Association of *Flavonifractor plautii*, a Flavonoid-Degrading Bacterium, with the Gut Microbiome of Colorectal Cancer Patients in India. *mSystems.* 2019;4(6):e00438-19.
- Karpat I, Karolyi M, Pawelka E, Seitz T, Thaller F, Wenisch C. *Flavonifractor plautii* bloodstream infection in an asplenic patient with infectious colitis. *Wien Klin Wochenschr.* 2021;133(13-14):724-72.
- Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiology Rev.* 2014;38: 996–1047.
- Steed AL, Christophi GP, Kaiko GE, Sun L, Goodwin VM, Jain U, Esaulova E, Artyomov MN, Morales DJ, Holtzman MJ, Boon ACM, Lenschow DJ, Stappenbeck TS. The microbial metabolite desaminotyrosine protects from influenza through type I interferon. *Science.* 2017;357(6350):498-502.
- Salminen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, Järvinen A. *Lactobacillus* bacteremia, clinical significance, and patient outcome, with special focus on probiotic *L. rhannosus* GG. *Clin Infect Dis.* 2004;38(1):62-9.
- Strachinaru DIC, Gallez JL, Daras S, Paridaens MS, Engel H, François PM, Rose T, Vanbrabant P, Soentjens P. A case of *Flavonifractor plautii* blood stream infection in a severe burn patient and a review of the literature. *Acta Clin Belg.* 2022;77(3):693-697.

Tourlousse DM, Sakamoto M, Miura T, Narita K, Ohashi A, Uchino Y, Yamazoe A, Kameyama K, Terauchi J, Ohkuma M, Kawasaki H, Sekiguchi Y. Complete Genome Sequence of Flavonifractor plautii JCM 32125. Microbiol Resour Announc. 2020;9(17):e00135-20.

Wilton A, Glezos CM, Pananwala H, Lim HK. Periprosthetic Hip Joint Infection with Flavonifractor plautii: A Literature Review and Case Report. Hip Pelvis. 2022;34(4):255-261. doi: 10.5371/hp.2022.34.4.255.

Yang G, Hong S, Yang P, Sun Y, Wang Y, Zhang P, Jiang W, Gu Y. Discovery of an ene-reductase for initiating flavone and flavonol catabolism in gut bacteria. Nat Commun. 2021;12(1):790.

5. Please describe the construction of the *C. glutamicum* production strain in more detail, including the following:

a. please state whether any antibiotic resistance genes are present or utilized during the construction of the production strain. Additionally, please clarify if the notifier has introduced or excised any other genes that are not described in the notice; if so, please briefly describe.

Daesang's response

Although the pDS-FDPE plasmid has the kanamycin resistance gene (*npt II*), it was used as a selection marker of the plasmid pDS-FDPE for constructing *Corynebacterium glutamicum* DS00001. However, the *nptII* gene is not utilized at any stage in the allulose production, and kanamycin is not added in any stages of fermentation.

Five genetic elements (*C. glutamicum* *sod* promoter, *nptII* promoter, FPDPE coding region, *nptII* coding region, and *C. glutamicum*-derived origin of replication) were introduced into the plasmid pDS-FDPE. The five genetic elements were *de novo* synthesized prior to inclusion. The *sod* promoter is originated from *Corynebacterium glutamicum* strain RES167 to drive the expression of FPDPE gene. The FPDPE gene is originated from *Flavonifractor plautii* to function as an expression of D-psicose 3-epimerase protein. The *nptII* promoter and *nptII* gene are originated from *E.coli* K-12 ER3413 to function as an expression kanamycin resistance protein. The *orfC/rep* is originated from *Corynebacterium glutamicum* to function as a replication origin. The origin, function and position of each gene are described at the below table.

Table 4. Introduced Genes in pDS-FDPE Plasmid

Gene	Origin	Function	Position in the plasmid (length)
<i>sod</i> promoter	<i>Corynebacterium glutamicum</i> strain RES167	Promoter (transcription start)	4448-4647 (200 bp)
FPDPE	<i>Flavonifactor plautii</i> ATCC 49531	D-psicose 3-epimerase	4648-5532 (885 bp)
<i>nptII</i> promoter	<i>E. coli</i> K-12 strain ER3413	Promoter (transcription start)	64-157 (94 bp)
<i>nptII</i>	<i>E. coli</i> K-12 strain ER3413	Kanamycin resistance	159-950 (792 bp)
<i>orfC/rep</i>	Replication protein (<i>C. glutamicum</i>)	Replication origin	2582-4045 (1464 bp)

Abbreviation: bp = base pairs.

b. please state whether the donor gene(s) are de novo synthesized or of bacterial origin

Daesang's response

The donor genes were *de novo* synthesized prior to inclusion in the expression construct.

c. please describe how the gene(s) were integrated into the *C. glutamicum* production strain.

Daesang's response

Corynebacterium glutamicum DS00001 was modified by inserting the pDS-FDPE plasmid. The *sod* promoter, originated from *Corynebacterium glutamicum* RES167, was amplified via PCR and inserted with *dpe* gene by fusion into the plasmid of site *PstI* and *BamHI*. In addition, the *nptII* promoter and *nptII* gene, originated from *E. coli* K-12 strain ER3413, were inserted into the plasmid of site *NdeI* and *NotI*. The individual gene origin of recombinant expression and Cleavage Map of pDS-FDPE plasmid are indicated in the below table and image.

Corynebacterium glutamicum DS00001 was constructed by inserting the pDS-FDPE plasmid into *C. glutamicum* ATCC 13032. The *sod* promoter derived from *Corynebacterium glutamicum* RES167 was inserted into the pDS-FDPE and the synthesized gene (*dpe*, FPDPE) coding for D- psicose 3-epimerase, originated from *Flavonifactor plautii* ATCC 49531, was introduced into the pDS-FDPE plasmid. To enable selection of the transformant of the pDS-FDPE, *nptII* promoter and *nptII* gene, originated from *E. coli* K-12, were inserted into the pDS-FDPE. The plasmid map showing the location of the introduced genes is shown in Figure 1. The resulting pDS-FDPE plasmid was transformed into the *C. glutamicum* ATCC 13032 competent cells using an

electroporation method. Then, the recombinant *Corynebacterium* cells were named *C. glutamicum* DS00001 (deposited as KCCM 80101).

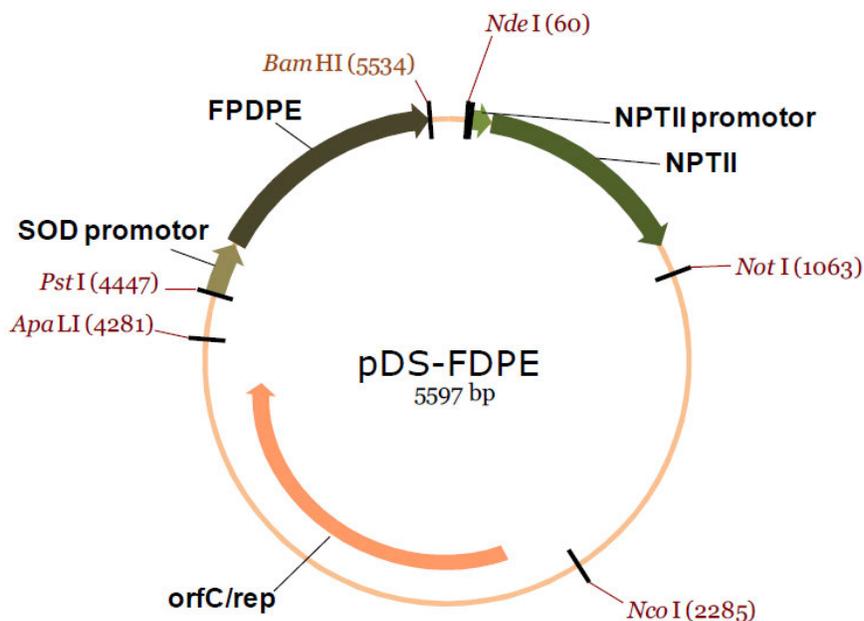


Figure 1. pDS-FDPE plasmid map

d. Please state how the integration of the gene(s) was confirmed d. please provide a narrative on the genetic stability of the *C. glutamicum* production strain and the ability of transfer of the introduced gene sequence(s)

How the integration of the gene(s) was confirmed.

Daesang's response

The plasmid pDS-FDPE is not integrated into the *C. glutamicum* DS00001 strain. As the plasmid pDS-FDPE contains an origin of replication, it can be stably maintained in *C. glutamicum*.

Please provide a narrative on the genetic stability of the *C. glutamicum* production strain

Daesang's response

Recombinant pDS-FDPE plasmid was transformed into *Corynebacterium glutamicum* ATCC13032, and the resulting transformant was designated as *Corynebacterium glutamicum* DS00001. The *dpe* gene, which functions as an expression of D-psicose 3-epimerase protein in pDS-FDPE plasmid, was designed not to be integrated in the host genome but to be inherited in

the plasmid. In order to verify the absence of non-intentional variations such as addition, insertion, or deletion of the plasmid DNA sequences during multiple generations, the *dpe* gene sequences of the plasmid were analyzed by sequencing plasmid DNA extracted from the culture of multiple generations.

After 20 subcultures, the *dpe* gene sequences in the isolated plasmid extracted from the 1st and 20th cultures were analyzed (Figure 2). The website used for homology search between the sequence is <http://uniprot.org>. As a result, there are no genetic mutations, deletions, or additions of the inserted *dpe* gene during cultures of the *C. glutamicum* DS00001 for multiple generations. All the *dpe* sequences from ATG, a start codon to TAA, a stop codon from the different cultures were 100% identical (Figure 3). In addition, when the strain loses the plasmid, D-psicose (D-allulose) production cannot proceed efficiently because the strain will also lose the enzyme protein expression, indicating the stability of *dpe* gene in the plasmid. Details of experimental conditions and results are presented below.

Sequencing primers

The recombinant pDS-FDPE plasmid was amplified with PCR primers listed in the Table below

Primers used in this study

Primer name	Sequence (5' → 3')
FDPE-F	CATAGGCAGATAACGGTTCCCCA
FDPE-R	GTTCAATCCCCATAACACCCCTTGT

Extraction and purification of plasmids

Recombinant pDS-FDPE plasmid was transformed into *Corynebacterium glutamicum* ATCC13032. A total of ten pDS-FDPE plasmid DNA samples were extracted from the 1st and 20th generations of culture of *C. glutamicum* DS00001 by using AccuPrep® Plasmid Mini Extraction kit (Bioneer, Korea)

Sequence analysis

The sequences of pDS-FDPE plasmid were verified by sequencing services provided by Bioneer (Korea). The sequences were analyzed by using BigDye Terminator v.3.1 sequencing kit and AB13730SL program (Applied Biosystems, USA).

Figure 2. Sequences from 1st, 20th culture of *C. glutamicum* DS00001

	Label	Blast		Length			GC%	Status	Re-reaction #	
		N	X	Normal	QV>16	QV>20				
1	1st_PDaseq5-R	-	-	1196	854	853	59.0		0	
2	1st_PDaseq5-F	-	-	1202	1049	898	61.0		0	
3	1st_PDaseq4-R	-	-	1147	1132	1070	57.0		0	
4	1st_PDaseq4-F	-	-	1195	708	415	56.0		1	
5	1st_PDaseq3-R	-	-	1133	1132	1072	59.0		0	
6	1st_PDaseq3-F	-	-	1104	812	813	59.0		0	
7	1st_PDaseq2-R	-	-	1179	899	899	54.0		0	
8	1st_PDaseq2a-F	-	-	1006	396	288	52.0		1	
9	1st_PDaseq1-R	-	-	1178	1177	1177	54.0		0	
10	1st_PDaseq1a-F	-	-	1167	1160	1075	53.0		0	
11	20th_PDaseq5-R	-	-	1149	1075	1075	59.0		0	
12	20th_PDaseq5-F	-	-	1176	823	660	61.0		0	
13	20th_PDaseq4-R	-	-	971	497	424	55.0		1	
14	20th_PDaseq4-F	-	-	997	268	267	55.0		1	
15	20th_PDaseq3-R	-	-	1145	1036	1021	59.0		0	
16	20th_PDaseq3-F	-	-	950	189	190	56.0		1	
17	20th_PDaseq2-R	-	-	1145	712	711	54.0		1	
18	20th_PDaseq2a-F	-	-	1033	613	606	50.0		1	
19	20th_PDaseq1-R	-	-	1194	886	889	55.0		0	
20	20th_PDaseq1a-F	-	-	1098	990	904	54.0		0	

Comparison of the *dpe* sequences in the plasmids isolated from the 1st, 20th cultures of *C. glutamicum* DS00001

All the *dpe* sequences in the plasmids isolated from 1st and 20th culture of *C. glutamicum* DS00001 were confirmed to be 100% identical, from ATG, a start codon to TAA, a stop codon (Figure 3). The website used for homology search between the sequences is <http://uniprot.org>.

FDPDE	1-----atgaaccggattggaatgcactacggcttctggagccacaactgggacga	50
day1-1	297TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	356
day1-2	300TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	359
day1-3	294TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	353
day1-4	301TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	360
day1-5	299TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	358
day20-1	297TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	356
day20-2	301TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	360
day20-3	299TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	358
day20-4	295TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	354
day20-5	294TTTTTACCCatgaaccggattggaatgcactacggcttctggagccacaactgggacga	353

FDPDE	51gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	110
day1-1	357gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	416
day1-2	360gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	419
day1-3	354gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	413
day1-4	361gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	420
day1-5	359gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	418
day20-1	357gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	416
day20-2	361gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	420
day20-3	359gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	418
day20-4	355gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	414
day20-5	354gattgcatacataccctgatggagaagctggcctggctgggctttgacatctgcgaggt	413

	
FDPDE	771taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	830
day1-1	1077taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1136
day1-2	1080taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1139
day1-3	1074taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1133
day1-4	1081taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1140
day1-5	1079taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1138
day20-1	1077taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1136
day20-2	1081taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1140
day20-3	1079taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1138
day20-4	1075taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1134
day20-5	1074taccattccctatgatatacaaggtctggcgggatctcagcggcggggccggggaggccgg	1133

FDPDE	831gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaa-----	885
day1-1	1137gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1196
day1-2	1140gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1199
day1-3	1134gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1193
day1-4	1141gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1200
day1-5	1139gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1198
day20-1	1137gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1196
day20-2	1141gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1200
day20-3	1139gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1198
day20-4	1135gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1194
day20-5	1134gctggacgagatggcgggcccgggctgcccgttccctcaaggagctgaccgcgtaaTGCCC	1193

Figure 3. Comparison DNA sequence between 1st and 20th cultures

The ability of transfer of the introduced gene sequence(s)

Daesang's response

The *C. glutamicum* DS00001 strain has kanamycin resistance because of the inserted antimicrobial resistance gene, *nptII*, which is originated from *E. coli* K-12, as a selection marker. To evaluate the ability of transfer of the *nptII* gene, the horizontal gene transfer frequency was tested by conjugation of *C. glutamicum* DS00001 and pathogenetic microorganisms.

This study was performed by filter-mating method. Specifically, the production strain, *C. glutamicum* DS00001 was used as a donor and twenty related and pathogenic rifampicin resistance-induced microorganisms were used as recipients. To discriminate the transconjugant from the donor and recipients, conjugants were grown with double antibiotic selection marker followed by colony selection. The donor and recipients were grown at their optimal growth condition for more than 12 hours. Donor and each recipient cell culture solutions were mixed in 1:8 (v/v) ratio and then incubated for 24 hours at each recipient's optimal conditions. The plates were grown in 30°C for 48 hours followed by colony counting. The conjugation frequency was estimated by the following equation.

$$\text{The conjugation frequency} = \frac{\text{The number of transconjugants}}{\text{The number of recipients}}$$

As a result, the horizontal gene transfer frequencies by conjugation among twenty tested microorganisms were below the detection limit ($<4.06 \times 10^{-10}$). These rates are equivalent to the minimum transfer frequencies of 1.3×10^{-9} derived from literature search. The test results indicate that the gene transfer by conjugation between the *C. glutamicum* DS00001 (or KCCM 80101) and twenty other microorganisms was not identified under conducted experimental conditions. Details are presented in Appendix D of this amendment.

6. For the administrative record, please briefly specify how the purity of the *C. glutamicum* production strain is ensured during manufacturing, and state whether the fermentation process is conducted in a contained, sterile environment.

Daesang's response

Fermentation process is carried out continuously through 1) pre-seed fermentation, 2) seed fermentation and 3) production fermentation to stably increase biomass at each step. Daesang has checked the purity of the culture strains by microscopic observation and/or agar plating after each of the three fermentation processes described above. And Daesang measured O.D. (Optical Density) every 4 hours during the fermentation process to monitor the culture strain's growth curve.

Daesang's manufacturing facility is certified for Food Safety System Certification 22000 (FSSC 22000). D-psicose is manufactured in compliance with the FSSC 22000 plan at a contained and sterilized facility and all culture media, containers, and fermenters are sterilized prior to introduction of *C. glutamicum* DS00001.

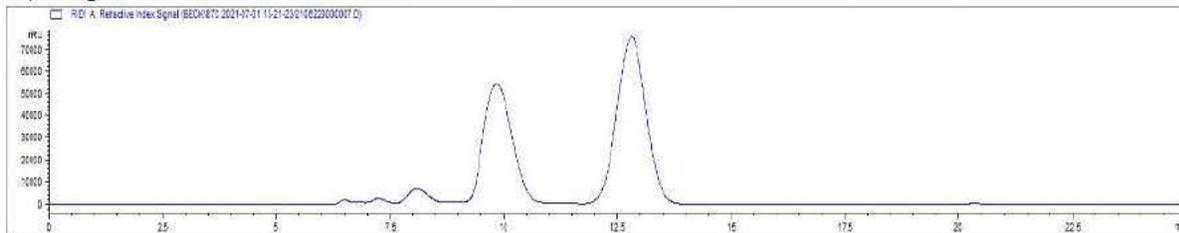
7. Please clarify if the enzyme, *D*-psicose 3-epimerase is intracellular and is not transported to the growth medium.

Daesang's response

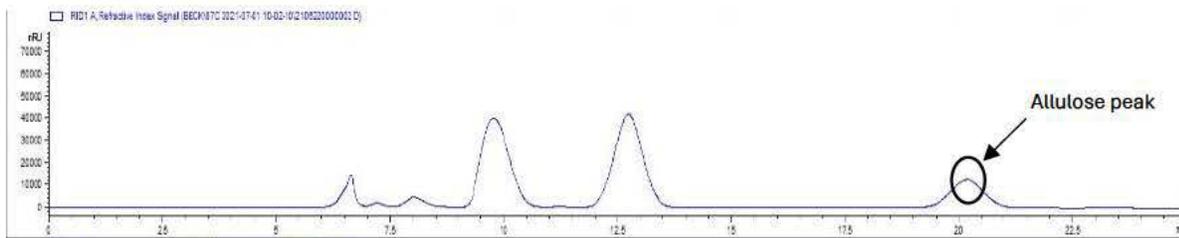
The enzyme *D*-psicose 3-epimerase is intracellular and is not released outside the bacterial cell. Daesang measured the enzyme reaction by HPLC with the culture supernatant to determine whether the enzyme was released outside the bacterial cell. No enzyme reaction was measured in the culture supernatant. In addition, a signal peptide is required for the enzyme protein to be released outside the bacterial cell, but the plasmid constructed by Daesang did not contain the signal peptide. Therefore, Daesang confirmed that the enzyme is not released outside the bacterial cell.

- HPLC test result : An allulose peak was found when the culture containing the bacterial cells was used for allulose production from fructose due to the intracellular enzyme reaction that converts fructose to allulose (as shown in result B below). But an psicose (allulose) peak was not found when only culture supernatant without the bacterial cells was used for allulose production from fructose as shown in result C below).

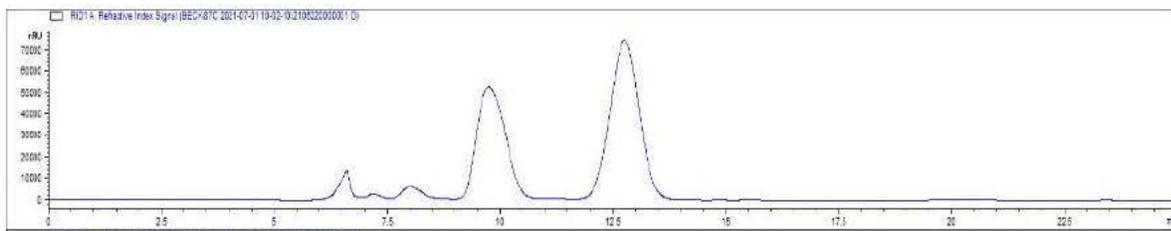
A) Negative Control – Fructose



B) Sample 1 – Culture solution



C) Supernatant culture solution



8. On page 14 (Table 2-2), antifoaming agent (polysorbate 80) is listed as one of the processing aids, but use of the antifoaming agent was not described in the manufacturing process. Please describe how polysorbate 80 is used as an antifoaming agent in the manufacturing process of D-psicose and address whether it is removed from the finished ingredient.

Daesang's response

Polysorbate 80 is used at the concentration of 3 parts per million of the culture for producing *C. glutamicum* DS00001 cells. After a series of purification steps using an activated carbon layer and ion exchange, polysorbate 80 is not expected to be present in the finished product. Under 21 CFR 172.840 polysorbate 80 is approved as an additive in yeast-defoamer formulations whereby the maximum amount of the additive in the yeast from such use does not exceed 4 parts per million.

9. For the administrative record, please state whether any of the raw materials used in the production of D-psicose are allergens or are derived from allergenic sources.

Daesang's response

No raw materials are allergens or are derived from allergenic sources.

10. Please state whether all analytical methods used to analyze the batches for conformance with the stated specifications have been validated for that particular purpose.

Daesang's response

Analytical values for specification conformance are based on analytical methods which have been validated for the intended purpose.

11. On pages 18-20, the notifier provides the results from the analyses of three batches to demonstrate that D-psicose can be manufactured to meet the proposed specifications. Please clarify if the results are from the analyses of non consecutive batches of D-psicose. In addition, please clarify if the results for heavy metals reported as "<[a value]" represent the limit of quantitation (LOQ) or the limit of detection (LOD) of the analytical method.

Daesang's response

The results are from the analyses of non-consecutive batches of D-psicose.

The results for heavy metals reported as "<[a value]" represent the limit of quantitation (LOQ). Please see Medallion Lab's COA in the attached at the end of this document (Appendix E)

12. In Part 2c, the notifier provides the specifications for D-psicose (Tables 3 and 4, pages 16-17).

a. In line with FDA’s “Closer to Zero” initiative that focuses on reducing dietary exposure to heavy metals, we recommend that the notifier establish lower specification limits for heavy metals that reflect the actual measured levels of heavy metals in the analyzed batches (Tables 5 and 6, pages 18-19) and are as low as possible.

b. Please consider including a limit for “total non-allulose saccharides” in the proposed specifications.

Daesang’s response

- a. Daesang agrees to lower the specifications for the heavy metals to ≤ 0.1 mg/kg.
- b. Daesang has included “total non-allulose saccharides” in the specifications.

A revised Tables 3 and 4 for specification are presented below.

Revised Table 3. Specifications of Daesang’s Crystalline D-psicose

Composition	D-psicose (D-allulose) crystal	
	Specifications	Methods of Analysis
Appearance	White powder	Visual
Odor	Odorless	-
Allulose*, g/100 g	≥ 98.0	HPLC
Total non-allulose saccharides*	< 2	HPLC
Moisture, g/100 g	< 5.0	AOAC 977.21
pH	3.0 – 7.0	pH meter
Ash, g/100 g	≤ 0.5	AOAC 900.02C
Pb, mg/kg	≤ 0.1	AOAC 2015.01
As, mg/kg	≤ 0.1	
Cd, mg/kg	≤ 0.1	
Aerobic plate counts, CFU/g	$\leq 1,000$	AOAC 990.12
Coliforms, CFU/g	< 10	AOAC 991.14
Yeast, CFU/g	≤ 100	AOAC 997.02

Composition	D-psicose (D-allulose) crystal	
	Specifications	Methods of Analysis
Mold, CFU/g	≤100	AOAC 997.02
<i>Salmonella</i> , CFU/25 g	Negative	AOAC 999.09

*Dry wt. basis

Revised Table 4. Specifications of Daesang's D-psicose Syrup

Composition	D-psicose syrup	
	Specifications	Methods of Analysis
Appearance	Light yellow liquid	Visual
Odor	Odorless	-
Allulose, g/100 g (dry wt. basis)	≥95	HPLC
Allulose, g/100 g (as-is basis)	≥65	HPLC
Total non-allulose saccharides	< 3	HPLC
Brix	≥63	Brix meter
Ash, g/100 g	≤0.5	AOAC 900.02C
Lead (Pb), mg/kg	≤0.1	AOAC 2015.01
Arsenic (As), mg/kg	≤0.1	
Cadmium (Cd), mg/kg	≤0.1	
Aerobic plate counts, CFU/g	≤1,000	AOAC 990.12
Coliforms, CFU/g	<10	AOAC 991.14
Yeast, CFU/g	≤100	AOAC 997.02
Mold, CFU/g	≤100	AOAC 997.02
<i>Salmonella</i> , CFU/25 g	Negative in 25 g	AOAC 999.09
<i>Staphylococcus aureus</i> , CFU/g	<10	AOAC 2001.05

Abbreviations: AOAC = Association of Official Analytical Community; CFU = colony forming units; HPLC = high-performance liquid chromatography.

13. In Tables 3 and 4 (page 16-17), the notifier lists the following specifications: a. Coliforms “however, on pages 68-73, the specification for coliforms is reported as “<10 CFU/g” ; however, on pages 68-73, the specification for coliforms is reported as “<10 CFU/10g” For the administrative record, please clarify the specification for coliforms. b. Salmonella serovars “Negative (CFU/25 g)”. For the administrative record, please confirm whether this refers to a specification of “negative/25 g”.

Daesang’s response

The specification for coliforms is <10 CFU/g.

We confirm that a specification for Salmonella is “negative/25 g”.

14. In Part 3 (Dietary Exposure), the notifier estimates an eaters-only cumulative dietary exposure to D-psicose from the current uses (GRNs 000400, 000498, 000623, and 000828) and intended uses of D-psicose to be 7.5 g/person (p)/d at the mean and 17.2 g/p/d at the 90th percentile for the U.S. population aged 2 years and older, using food consumption data from the 2017-2018 National Health and Nutrition Examination Survey (NHANES) (Table 12-1, page 28).

FDA has performed a cumulative dietary exposure assessment for D-psicose based on the food codes (Appendix D) and corresponding use levels (Table 1, page 9) using the 2017-2018 NHANES, and our results are significantly higher than those the notifier reported in GRN 001148.

We suggest that the notifier address the cumulative dietary exposure to D-psicose by providing the following information:

- a. We note that the recent GRAS notices (GRNs 001024, 001029, and 001057) for D-psicose that received “no questions” letters were not included in Table 10 (pages 25-26). In addition, we note that uses of D psicose in “Alcoholic beverages (premixed cocktails, wine coolers, and malt beverages, low/reduced calorie)” as well as in “Nutrition bars, cereal based bars, and protein bars” were included in GRN 001024 and GRN 001057, respectively. For completeness of the record, please provide an updated Table 10 including the uses of D-psicose notified in GRNs 001024, 001029, and 001057.**

Daesang’s response

Daesang has revised its intended use and use levels to the cumulative use levels derived from previous GRAS notices except for jerky. Daesang proposes to use D-psicose (D-allulose) as a food ingredient (a sugar substitute/sweetener) at use levels ranging from 2.1 to 100%. Daesang proposes to use D-psicose (D-allulose) as a food ingredient in selected foods listed in Revised

Table 1: bakery products (rolls, cakes, pies, pastries, and cookies: low calorie or dietetic); low-calorie beverages; selected cereals; chewing gums; coffee mix; confections and frostings; dressings for salads; fat-based creams; frozen dairy desserts (ice cream, soft serve, and sorbet: low-calorie, reduced-calorie, and sugar-free); gelatins, pudding, and fillings (low-calorie, reduced-calorie, and sugar-free); hard candies (low-calorie); soft candies (non-chocolate, plain chocolate, and chocolate coated; low-calorie, reduced-calorie, and sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low-calorie, reduced-calorie, and sugar-free); yogurt (regular and frozen: low-calorie, reduced-calorie, and sugar-free); nutrition bars, grain-based cereal bars, and protein bars; and alcoholic beverages (premixed cocktails, wine cooler, hard seltzer, and malt beverage: low- or reduced-calorie and low-or reduced-sugar), nutritional beverages, nutritional beverages for children, ketchup and barbeque sauce, and dried cranberry.

Revised Table 1. Intended Use and Maximum Use Levels of D-psicose (D-allulose), % (w/w)

Description	GRN 001148 Daesang
Bakery products (rolls, cakes, pies, pastries, and cookies) rolls, cakes, pastries, cakes, low calorie or dietetic	10
Beverages, carbonated: low calorie	3.5
Beverages (non-alcoholic) low calorie, reduced calorie, sugar-free)	2.1
Cereals, ready to eat (<5% sugar)	10
Cereals, RTE grain-free, no-sugar, no protein	20
Cereals, low-and reduced calorie, sugar free	12
Cereals, regular	12
Chewing gum	50
Coffee mix	30
Confections and frostings	5
Dressings for salads	5
Fat-based cream - used in modified fat/calorie cookies, cakes, pastries and pie	10
Frozen dairy desserts (ice cream, soft serve, sorbet: low calorie, reduced calorie, sugar free)	8
Gelatins, pudding, fillings (low calorie, reduced calorie, sugar free)	10
Hard candies (low calorie)	70
Soft candies (non-chocolate, plain chocolate, chocolate coated) (low calorie, reduced calorie, sugar free)	25
Jams and jellies	10

Sugar	10
Sugar substitutes	100
Sweet sauces and syrups low calorie, reduced calorie and sugar free	10
Yogurt (regular and frozen), low calorie, reduced calorie, sugar free	5
Nutrition bar, grain-based cereal bars, protein bars	15
Alcoholic beverages (premixed cocktails, wine cooler, hard seitzer, and malt beverage), low- or reduced calorie; low-or reduced sugar	3.5
Nutritional beverages	2.5
Nutritional beverages for children	3.5
Ketchup and barbeque sauce	10
Cranberry, dried	25

- a. In Appendix C, we believe there is a typographical error in the use level for alcoholic beverages and it should be a 3.5% indicated as Tables 1 and 10, and not a 4%. Please clarify this discrepancy.*

We apologize for the typo. The intended use level of alcoholic beverages should have been 3.5%.

- b. Please confirm that the intended uses of D-psicose notified in GRN 001148 are substitutional for the uses notified in previous GRNs for D-psicose and therefore, the cumulative dietary exposure to D-psicose reported in GRN 001057 will not increase as a result of the intended uses of D-psicose notified in this GRAS notice, GRN 001148. In the notifier's response, please also incorporate by reference the cumulative dietary exposure estimates from GRN 001057.*

Daesang's revised intended use and use levels are same as the cumulative use levels described in previous GRAS notices except for jerky (jerky is not included in GRN001148). Revised Table 10, Psicose (Allulose) Cumulative Intended Use and Use Levels from the Previous GRAS Notices, is presented below.

The intended uses of D-psicose notified in GRN 001148 are substitutional for the uses notified in previous GRNs for D-psicose and therefore, there will be no increase in the cumulative dietary exposure to D-psicose, i.e., the cumulative dietary exposure to D-psicose reported in GRN 001057 will not increase as a result of the intended uses of D-psicose notified in GRN 001148. Thus, this notice incorporates, by reference, the cumulative dietary exposure estimates from GRN 001057. Briefly, the mean and 90th percentile daily intakes are estimated to be 14.5

and 29 g, respectively, in all-users aged 2 years and above (a pseudo 90th percentile intake was estimated by multiplying the mean value by 2 in this exposure estimate). Thus, the intended uses of D-psicose notified in GRN 001148 are expected not to exceed 29 g/person/day in all-users aged 2 years and above.

Revised Table 10. Psicose (Allulose) Cumulative Intended Use and Use Levels from the Previous GRAS Notices

Description	GRN 000400	GRN 000498	GRN 000693	GRN 000828	GRN 001024	GRN 001029	GRN 001057	Cumulative Use levels from previous 7 GRNs*	GRN 001148
Bakery products (rolls, cakes, pies, pastries, and cookies) rolls, cakes, pastries, cakes, low calorie or dietetic	10	NA	10	10	10	10	NA	10	10
Beverages, carbonated, low calorie	2.1	3.5	3.5	NA	3.5	2.1	NA	3.5	3.5
Beverages, non-carbonated (non-alcoholic) low calorie, reduced calorie, sugar-free)	2.1	3.5	3.5	3.5	3.5	2.1	NA	3.5	2.1
Cereals, ready to eat (<5% sugar)	10	NA	NA	NA	10	10	NA	10	10
Cereals, low-and reduced calorie, sugar free	NA	5	5	5	5	NA	12	12	12
Cereals, regular	NA	2	2	2	2	NA	12	12	12
Cereals, RTE, grain-free, no sugar, no protein	NA	NA	NA	NA	NA	NA	20	20	20
Chewing gum	50	50	50	50	50	50	NA	50	50
Coffee mix	30	NA	NA	NA	30	30	NA	30	30
Confections and frostings	NA	5	5	5	5	NA	NA	5	5
Dressings for salads	NA	5	5	5	5	NA	NA	5	5
Fat-based cream - used in modified fat/calorie cookies, cakes, pastries and pie	10	NA	5	5	10	10	NA	10	10

Frozen dairy desserts (ice cream, soft serve, sorbet: low calorie, reduced calorie, sugar free)	5	5	5	5	5	5	8	8	8
Gelatins, pudding, fillings (low calorie, reduced calorie, sugar free)	NA	10	10	10	10	NA	NA	10	10
Hard candies (low calorie)	70	50	50	50	50	70	NA	70	70
Soft candies (non-chocolate, plain chocolate, chocolate coated) (low calorie, reduced calorie, sugar free)	25	25	25	25	25	25	NA	25	25
Jams and jellies	NA	10	10	10	10	NA	NA	10	10
Sugar	NA	10	10	10	10	NA	NA	10	10
Sugar substitutes	100	100	100	100	100	100	NA	100	100
Sweet sauces and syrups low calorie, reduced calorie and sugar free	NA	10	10	10	10	NA	NA	10	10
Yogurt (regular and frozen), low calorie, reduced calorie, sugar free	5	5	5	5	5	5	NA	5	5
Nutrition bar, grain-based cereal bars, protein bars	NA	NA	NA	NA	NA	NA	15	15	15
Alcoholic beverages (premixed cocktails, wine cooler, hard seltzer, and malt beverage), low- or reduced calorie; low-or reduced sugar	NA	NA	NA	NA	3.5	NA	NA	3.5	3.5
Nutritional beverages	NA	NA	NA	NA	NA	NA	2.5	2.5	2.5
Nutritional beverages for children	NA	NA	NA	NA	NA	NA	3.5	3.5	3.5
Ketchup and barbeque sauce	NA	NA	NA	NA	NA	NA	10	10	10

Cranberry, dried	NA	NA	NA	NA	NA	NA	25	25	25
Jerky, meat- and poultry-based	NA	NA	NA	NA	NA	NA	15	15	NA

Abbreviation: NA = not applicable.

Estimated Daily Intake (CEDI) of D-psicose

Subpopulation	CEDI (g/day)		
	Mean per capita	Mean per user*	Pseudo 90 th ** per user
2+ yr	11.2	14.5	29.0
2-12 yr	7.5	10.6	21.2
13-18 yr	6.9	10.9	21.8
19+ yr	12.3	15.1	30.2

Cumulative EDIs (from previous GRAS notices) were adopted from GRN 001057 amendment page 3 of the Revised Cumulative Estimated Daily Intake of Allulose (or page 196 of the entire document).

*Mean per capita divided by % users to derive mean per user.

**Pseudo 90th is derived based on 2 x mean (FDA 2006 guidance).

Abbreviations: CEDI = cumulative estimated daily intake

15. The notifier provides a safety narrative in Part 6 of the GRAS notice (page 35).

a. Please provide an updated literature search including the date (month and year) the literature search was performed and discuss the identity and safety of *C. glutamicum*.

Daesang's response

a. The literature search was done to cover the literature published until February, 2024.

Identity and safety of C. glutamicum

Daesang's production strain *C. glutamicum* DS00001 is a genetically modified derivative of the host strain *C. glutamicum* ATCC 13032. The production strain DS00001 has been deposited at the KCCM.

The strain *Corynebacterium glutamicum* ATCC 13032 is a non-pathogenic and non-sporulating gram-positive soil bacterium which belongs to the order *Actinomycetales*. This microorganism has a long history of applications in the production of various amino acids and other industrially relevant compounds. The complete genome sequence of the representative wild-type strain of *C. glutamicum* ATCC 13032 has been determined (Ikeda and Nakagawa, 2003; Kalinowski et al. 2003). The genome sequence comprises a circular chromosome with a length of almost 3.3 Mb and harbors more than 3,000 annotated protein coding sequences (CDS).

C. glutamicum ATCC 13032 has been widely used in the production of many safe food ingredients including D-psicose (GRN 693; FDA, 2017, by Samyang Corporation), and 2-fucosyllactose (GRN 932; FDA 2021, by Advanced Protein Technologies Corporation [APTech]). In addition, the literature reports the safe use of *C. glutamicum* ATCC 13032 in the production of many amino acids, such as L-lysine (Kim et al., 2013), L-citrulline (Hao et al., 2015), and L-theanine (Ma et al., 2020).

In the USA, the safe use of other *C. glutamicum* strains in the food ingredients is described in many GRAS notices including, but not limited to, D-psicose (GRN 400; FDA 2012a, by CJ CheilJedang), L-lysine monohydrochloride (GRN 414; FDA 2012b, by Procter & Gamble Company), L-leucine (GRN 523; FDA 2014, by Innobio Limited), and cultured corn starch (GRNs 792; FDA 2019, by Nestec, S.A. [Nestle]).

In Europe, the bacterial species *C. glutamicum* has been recommended for the qualified presumption of safety (QPS) status for production purposes (EFSA Scientific Committee, 2007, pages 3-4; EFSA BIOHAZ Panel, 2024). The QPS process was developed to provide a harmonized safety assessment approach based on the taxonomic identity, body of relevant knowledge and safety of microorganisms. Under the QPS system, a safety assessment of a defined taxonomic group (e.g. genus or group of related species) could be made based on four pillars (establishing identity, body of knowledge, possible pathogenicity, and end use). Thereafter, any strain of microorganism the identity of which could be unambiguously

established and assigned to a QPS group would be freed from the need for further safety assessment other than satisfying any qualifications specified. A generic qualification for all QPS bacterial taxonomic units applies in relation to the absence of acquired genes conferring resistance to clinically relevant antimicrobials. In relation to the QPS status of *Corynebacterium glutamicum*, the EFSA Scientific Committee (2007) states the following: “*Corynebacterium glutamicum* plays an important role in the amino acid fermentation industry. No safety concerns are reported for this bacterial species for humans and animals, and no information on the presence of acquired antibiotic resistances in this bacterial species is available. However, it should be kept in mind that the direct exposure of consumers to this bacterial species is expected to be very low. --- *C. glutamicum* has generally been considered to be non-pathogenic and no safety concerns are envisaged.” EFSA BIOHAZ Panel (2024; Table 1 of the Panel report) confirmed the QPS status of *C. glutamicum*.

Overall, the history of safe use of *C. glutamicum* ATCC 13032 in the production of many food ingredients as described in previous GRAS notices and the literature and the QPS status of *C. glutamicum* established by the EFSA committees support the safety of *C. glutamicum* for production purposes.

Equally importantly, no viable cells and residual DNA (quantified by polymerase chain reaction [qPCR]) from the production strain were detected in the finished ingredient D-psicose.

References

EFSA Scientific Committee, 2007. Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. EFSA Journal 2007;5(12):587, 16 pp. <https://doi.org/10.2903/j.efsa.2007.587>.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2024. Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 19: Suitability of taxonomic units notified to EFSA until September 2023. EFSA J. 2024;22:e8517.

Food and Drug Administration (FDA). 2012a, FDA GRN 000400. D-psicose, filed by CJ CheilJedang. Available at: [UCM277372.pdf \(archive-it.org\)](#).

FDA 2012b, GRN 000414. L-lysine monohydrochloride, filed by Proctor & Gamble Co. Available at: [UCM299316.pdf \(archive-it.org\)](#).

FDA 2014, GRN 000523. L-leucine, filed by InnoBio Limited. Available at: [UCM407679.pdf \(archive-it.org\)](#).

FDA 2017, 000693. D-psicose, filed by Samyang Corporation. Available at: [download \(fda.gov\)](#).

- FDA 2019, GRN 000792. cultured corn starch, filed by Nestec, S.A. (Nestle). Available at: [download \(fda.gov\)](#).
- FDA 2021, 000932. 2'-fucosyllactose, filed by Advanced Protein Technologies Corporation [APTech]. Available at: [download \(fda.gov\)](#).
- Hao N, Mu J, Hu N, Xu S, Yan M, Li Y, Guo K, Xu L. Improvement of L-citrulline production in *Corynebacterium glutamicum* by ornithine acetyltransferase. *J Ind Microbiol Biotechnol*. 2015;42(2):307-13.
- Ikeda M, Nakagawa S. The *Corynebacterium glutamicum* genome: features and impacts on biotechnological processes. *Appl Microbiol Biotechnol*. 2003;62(2-3):99-109.
- Kalinowski J, Bathe B, Bartels D, Bischoff N, Bott M, Burkovski A, Dusch N, Eggeling L, Eikmanns BJ, Gaigalat L, Goesmann A, Hartmann M, Huthmacher K, Krämer R, Linke B, McHardy AC, Meyer F, Möckel B, Pfefferle W, Pühler A, Rey DA, Rückert C, Rupp O, Sahm H, Wendisch VF, Wiegräbe I, Tauch A. The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. *J Biotechnol*. 2003;104(1-3):5-25.
- Kim HI, Nam JY, Cho JY, Lee CS, Park YJ. Next-generation sequencing-based transcriptome analysis of L-lysine-producing *Corynebacterium glutamicum* ATCC 21300 strain. *J Microbiol*. 2013;51(6):877-80.
- Ma H, Fan X, Cai N, Zhang D, Zhao G, Wang T, Su R, Yuan M, Ma Q, Zhang C, Xu Q, Xie X, Chen N, Li Y. Efficient fermentative production of L-theanine by *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol*. 2020;104(1):119-130.

b. When discussing previous, related GRAS notices evaluated by FDA that received “no questions” response letters, the notifier mentions GRN 001024 on page 35, the subject of which was D-psicose as a sweetener in various foods but does not include it in Table 15 on the same page. Further, the notifier does not include GRNs 001029 and 001057, the subjects of which were both D-psicose as a sweetener in various foods. For the administrative record, please briefly discuss whether these GRAS notices affect the notifier’s GRAS conclusion.

Daesang’s response

Daesang has revised Table 15 to include use levels described in GRNs 001024, 001029, and 001057.

The status of previous GRAS notices does not affect Daesang’s GRAS conclusion because Daesang intended use and use levels are cumulative intended use levels from previous GRAS notices except for jerky. An updated Table 15 is shown below.

Revised Table 15. Summary of GRAS Notices in the U.S.

GRN	Company	Production microorganism (enzyme source)	Intended use	EDI, 90 th pctl in all users
Current notice				
001148	Daesang	GM <i>C. glutamicum</i> (<i>Flavonifractor plautii</i>)	As a sweetener in various foods at use levels ranging from 2.1 to 100% (cumulative use from GRN 400, 498, 623, and 828, 1024, 1029, and 1057 except jerky)	Up to 29.0 g/p/d (NHANES 2017-2018)
Previous GRAS notices				
001057	Tate & Lyle	Alpha-amylase from <i>Aspergillus oryzae</i> , glucoamylase from <i>A. niger</i> , glucoisomerases from GM <i>Streptomyces rubiginosus</i> , and D-allulose 3-epimerase from GM <i>E. coli</i> K12 (enzyme source, NA)	As a sweetener in various foods at use levels ranging from 2 to 100%	11.9 g/p/d from the intended use; cumulative EDI of 29.0 g/p/d (NHANES 2017-2018)
001029	L & P Food Ingredient Co., Ltd.	Non-GM <i>B. subtilis</i> possessing D-allulose 3-epimerase activity	As a sweetener in various foods at use levels ranging from 2.1 to 100%	17.6 g/p/d (NHANES 2017-2018)
001024	Blue California	GM <i>E. coli</i> JM109 (enzyme source, NA)	As a sweetener in various foods at use levels ranging from 2.1 to 100%	22.9 g/p/d (NHANES 2017-2018)
000828	Samyang Corp.	Non-GM <i>Microbacterium foliorum</i> harboring D-allulose 3-epimerase activity	As a sugar substitute in food applications at use levels ranging from 2 to 100%	30 g/p/day or 0.42 g/kg bw/day (NHANES 2007-2010)
000693	Samyang Corp.	GM <i>C. glutamicum</i> (<i>Clostridium scindens</i>)		
000498	Matsutani	GM <i>Escherichia coli</i> (K12) or <i>Streptomyces violaceoruber</i>	As a sugar substitute in food applications at use levels ranging from 2 to 100%	24.8 g/p/day or 0.33 g/kg bw/day

		(<i>Arthrobacter globiformis</i>)		
000400	CJ CheilJedang	GM <i>C. glutamicum</i> (<i>Arthrobacter globiformis</i>)	As a sugar substitute in foods at use levels ranging from 2 to 10%	28.5 g/p/day or 0.36 g/kg bw/day

Enzyme = D-psicose-3-epimerase.

Abbreviations: bw = body weight; g/p/d = g/person/day; GRN = Generally Recognized as Safe notice; GM = genetically modified; NA = not available; pctl = percentile.

*The 90th percentile EDIs would be 25-33 g/p/d or 0.34-0.46 g/kg bw/day if the NHANES 2007-2010 dataset would be employed in exposure estimates.

The pertinent information is available as indicated below:

GRN 000400 (FDA, 2011):

<http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=400>.

GRN 000498 (FDA, 2012):

<http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=498>.

GRN 000693 (FDA, 2017):

<http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=693>.

GRN 000828 (FDA, 2019):

<https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=828>.

GRN 001024 (FDA 2023a): D-psicose, filed by Blue California. [GRAS Notices \(fda.gov\)](#).

GRN 001029 (FDA 2023b): D-psicose, filed by L&P Food Ingredient Co., Ltd. [download \(fda.gov\)](#).

GRN 001057 (FDA 2024): D-psicose, filed by Tate & Lyle. [download \(fda.gov\)](#)

We hope we properly responded to FDA questions. Please let me know if there are any further clarifications required. Thank you in advance for your kind attention to this amendment.

Sincerely,

Susan

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STUDY REPORT

Bioinformatic analysis of *Corynebacterium glutamicum* KCCM 80101 based on whole genome sequencing data

Author (s)	Technical review
[Redacted]	[Redacted]
	Approved by
	[Redacted]

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This report contains scientific interpretation of the received data by the named scientists in the Biosafe Ltd. It does not necessarily represent the official views of the competent authorities.

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58 EXECUTIVE SUMMARY

59 **Scope:** The company Daesang Corp requested Biosafe Ltd to provide a whole genome sequencing-based
60 bioinformatics analysis for the evaluation of *Corynebacterium glutamicum* KCCM 80101 (DS 00001),
61 hereafter 'the test strain', according to EFSA guidance on the characterisation of microorganisms used as
62 feed additives or as production organisms and EFSA statement on the requirements for whole genome
63 sequence analysis of microorganisms intentionally used in the food chain (EFSA 2018, 2021). The strain is
64 genetically modified to enable the production of allulose.

65 **Methods:** Biosafe received the production strain from manufacturer. DNA extraction was carried out at
66 Biosafe. Sequencing services was provided by Eurofins Genomics. The test strain was sequenced using
67 Illumina and Nanopore sequencing technologies. Taxonomic identification was based on alignment and
68 non-alignment tools using the whole genome sequence data. The assembly consisted of 2 contigs
69 producing complete genome for this bacterial species. Genes of concern and genetic modifications were
70 searched from the genome according to the guidance documents.

71 **Results:** Based on 16S rRNA and whole genome-based analyses, the test strain is a member of the species
72 *Corynebacterium glutamicum*.

73 **Antimicrobial resistance genes:** Two antimicrobial resistance genes (*cmr*, *npt II*) exceeded the EFSA
74 thresholds for identity and coverage. The minimum inhibitory concentration (MIC) analysis for the strain
75 indicated that the test strain is susceptible to ampicillin, gentamicin, kanamycin, streptomycin, tetracycline,
76 erythromycin, clindamycin, chloramphenicol, and vancomycin against *Corynebacterium glutamicum* KCCM
77 80101 was analysed.

78 **Genetic modifications:** The genome is modified for allulose production.

79 **Mobile genetic elements:** One plasmid sequence was identified in the test strain. A total of 34 insertion
80 sequences and one potential phage sequence region was detected in the test strain genome. Mobile genetic
81 elements were not linked to the antimicrobial resistance gene.

82 **Conclusion:** The test strain is a member of the species *Corynebacterium glutamicum* and it is closely related
83 to the strain *Corynebacterium glutamicum* ATCC 13032. Two antimicrobial resistance genes exceeded the
84 identity and coverage thresholds. Gene *cmr*, which encodes a multi-drug efflux MFS transporter protein of
85 *Corynebacterium glutamicum* and is intrinsic and not associated with mobile genetic elements. The gene *npt*
86 *II*, which was included as a part of the genetic modification. *Npt II* encodes aminoglycoside 3'-
87 phosphotransferase and inactivates aminoglycoside antibiotics like kanamycin and is used widely as a
88 selectable marker. The MIC-values for ampicillin, streptomycin, tetracycline, erythromycin, clindamycin,
89 chloramphenicol, and vancomycin against *C. glutamicum* KCCM 80101 were equal or below the EFSA MIC
90 cut-off values, indicating that the strain is susceptible to these antimicrobials. The MIC value for kanamycin
91 exceeded eight-fold the EFSA cut-off values for *Corynebacterium* (Biosafe report T1493R1502/2022). The
92 genetic modifications enable the production of allulose. . The MIC value for kanamycin exceeded eight-
93 fold the EFSA cut-off values for *Corynebacterium* and other Gram-positive bacteria (Biosafe report
94 T1493R1502/2022).

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98 1. INTRODUCTION

99 The company Daesang Corp requested Biosafe Ltd to provide a whole genome sequencing-based
100 bioinformatics analysis for the evaluation of *Corynebacterium glutamicum* KCCM 80101 (DS 00001),
101 hereafter 'the test strain', according to EFSA guidance on the characterisation of microorganisms used as
102 feed additives or as production organisms and EFSA statement on the requirements for whole genome
103 sequence analysis of microorganisms intentionally used in the food chain (EFSA 2018, 2021). The purpose
104 of this report is to evaluate the data against the requirements of the guidance. The strain is genetically
105 modified to enable the production of allulose.

106 2. DATA AND METHODS

107 2.1. WGS data and contamination analysis

108 Biosafe received the production strain from the client (**Table 1**). Sequencing was performed using Illumina
109 and Nanopore technologies and provided by Eurofins GmbH where Biosafe received raw data (**Table 2**). The
110 minimum inhibitory concentration analysis of selected antimicrobials against the test strain was done at
111 Biosafe (Biosafe report T1493R1502/2022).

112 **Table 1:** Significant dates in the project

Sample code within this document	Strain	Form of arrival	Biosafe sample code	Date
Test strain	<i>C. glutamicum</i> DS 00001 (KCCM 80101)	freeze-dried	379/22_1-2	15.8.2022 (arrival)
Notified study start date				19.9.2022
DNA extraction				21.9.2022
Nanopore data received from sequencing				7.11.2022
Illumina data received from sequencing				10.10.2022
Bioinformatic analysis start date				5.12.2022
Notified study end date				31.12.2022

113 **Table 2.** Description of the WGS and assembly

Description of the genome		Test strain
Raw reads FASTQ	Illumina	NG-31379_T1493_379_22_lib636260_10107_3_1.fastq
	Nanopore	IC-2514_1_lib637180_T1493_379_Nanopore_FAV08399_pass_all.fastq
Assembly name		The test strain
Raw reads (n)	Nanopore	27,208
	Illumina	9,429,410 (pair-ended)
Sequenced bases (bp)	Nanopore	428,624,366
	Illumina	2,847,681,820
Reads recognized by the contamination tool	Illumina	9,429,410
	Nanopore	27,208
Classified to unexpected organism	Illumina	270,628 (2.87%)
	Nanopore	38 (0.14%)
Assembly length		3,282,549
Assembly N50		3,282,549
Assembly CG%		53.8

114 No more than 5% of reads were assigned to an unexpected organism (**Tables 3 and 4**). No reads were
 115 assigned to organisms which would indicate contamination. The sequencing coverage for the illumina and
 116 nanopore data was 868x and 131x respectively, which fulfils the EFSA requirement that greater than 30x
 117 coverage is achieved. The assembly N50 value was 3,282,540 and the total assembly size was 100.2% of
 118 the size of the parent strain which fulfils the EFSA requirement that the assembly is within 20% of the
 119 expected genome size.

120 **Table 3.** Results of the taxonomy classification for contamination check for illumina sequencing. Number
 121 of assigned reads is the number assigned directly to the class. *Rank code indicates (U)nclassified, (D)omain,
 122 (K)ingdom, (P)hylum, (C)lass, (O)rder, (F)amily, (G)enus, or (S)pecies. Organisms with with less than 0.01%
 123 reads assigned to them were filtered out for simplicity.

% of Reads	Number of Reads	Reads (n) assigned to this taxon	Rank code*	NCBI Tax. ID	Scientific name
3.46	326231	326231	U	0	unclassified
96.54	9103179	0	R	1	root
96.54	9103179	8693	R1	131567	cellular organisms
96.45	9094485	133010	D	2	Bacteria
95.02	8959667	2311	D1	1783272	Terrabacteria group
93.87	8851826	44	P	201174	Actinobacteria
93.87	8851782	17052	C	1760	Actinobacteria
93.69	8834720	1716	O	85007	Corynebacteriales
93.67	8832992	441	F	1653	Corynebacteriaceae
93.67	8832551	4597367	G	1716	Corynebacterium
44.88	4231891	4230789	S	1718	Corynebacterium glutamicum
0.01	776	776	S1	340322	Corynebacterium glutamicum R
0.02	2262	2262	S	401472	Corynebacterium ureicelerivorans
1.12	105526	1	P	1239	Firmicutes
1.12	105514	0	C	186801	Clostridia
1.12	105514	10	O	186802	Clostridiales
1.12	105504	0	F	541000	Ruminococcaceae
1.12	105504	0	G	946234	Flavonifractor
1.12	105504	105504	S	292800	Flavonifractor plautii
0.02	1806	1476	P	1224	Proteobacteria

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125 **Table 4.** Results of the taxonomy classification for contamination check for Nanopore sequencing. Number
 126 of assigned reads is the number assigned directly to the class. *Rank code indicates (U)nclassified, (D)omain,
 127 (K)ingdom, (P)hylum, (C)lass, (O)rder, (F)amily, (G)enus, or (S)pecies. Organisms with less than 0.01% reads
 128 assigned to them were filtered out for simplicity.

% of Reads	Number of Reads	Reads (n) assigned to this taxon	Rank code*	NCBI Tax. ID	Scientific name
0.04	10	10	U	0	unclassified

99.96	27198	0	R	1	root
99.96	27198	0	R1	131567	cellular organisms
99.96	27198	8	D	2	Bacteria
99.93	27190	6	D1	1783272	Terrabacteria group
99.88	27174	0	P	201174	Actinobacteria
99.88	27174	14	C	1760	Actinobacteria
99.82	27160	0	O	85007	Corynebacteriales
99.82	27160	0	F	1653	Corynebacteriaceae
99.82	27160	17380	G	1716	Corynebacterium
35.95	9780	9780	S	1718	Corynebacterium glutamicum
0.04	10	0	P	1239	Firmicutes
0.04	10	0	C	186801	Clostridia
0.04	10	0	O	186802	Clostridiales
0.04	10	0	F	541000	Ruminococcaceae
0.04	10	0	G	946234	Flavonifractor
0.04	10	10	S	292800	Flavonifractor plautii

129

130 Methods

131 Methods used to analyze the whole genome sequence are described in **Appendix 1 (confidential)**.

132 3. RESULTS AND DISCUSSION

133 3.1. General description of the genome

134 The description of the test strain genome is shown in **Table 5**. Comparison of the test strain with the data
135 available on GenBank is also shown.

136 The annotated genome will be provided to the client in genbank-format (.gb).

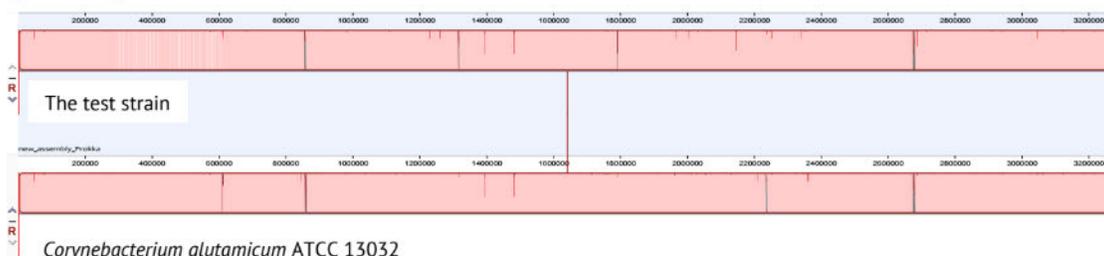
137 **Table 5.** Description of the test strain genome and comparison to *Corynebacterium glutamicum*. NA, not
138 available.

Description of the test strain genome	Test strain	Data for <i>Corynebacterium glutamicum</i> from NCBI GenBank	Data for <i>Corynebacterium glutamicum</i> ATCC 13032 parent strain
Search date	NA	07/12/2022	07/12/2022
Available genomes	NA	75	1
Number of contigs	2	NA	1
Total size of the annotated genome	3,288,137	3,265,960 bp	3,282,708
%CG	53.8	54.1	53.8
Genes	3,128	NA	3,046
CDSs	3,028	2,854	2,966
rRNAs	18	NA	18
tRNAs	60	NA	59
tmRNA	1	NA	1

miscellaneous RNAs	21	NA	NA
Plasmids	1	NA	None

139 3.2. Overall organisation of the genome: whole genome alignment

140 A closely related genome was selected for pairwise comparison based on the taxonomic identification
141 results described in Section 3.4. The comparison by whole genome alignment aims to assess the genome
142 completeness and organisation of the test strain. The assessment was made against the genome of the
143 reported parental strain *Corynebacterium glutamicum* ATCC 13032 (**Figure 1**). The visualization of the whole
144 genome alignment indicates that the genome assembly of the test strain has good congruence with the
145 parental genome.



146

147 **Figure 1.** Whole genome alignment of the test strain (top) and the genome of *Corynebacterium glutamicum*
148 ATCC 13032 (bottom). Matches between the genomes are shown as linked boxes (similarly colored blocks).
149 Contig boundaries are indicated using red vertical lines.

150 3.3. Screening for mobile genetic elements

151 Contig 2 was identified as the complete plasmid and no additional plasmid sequences were found in the
152 test strain (**Table 6**). A total of 34 transposons (insertion sequences) and one potential phage sequence
153 were detected in the test strain (**Table 7**). Findings were not associated with the genes of concern.

154 **Table 6.** Detected plasmid on the test strain.

Contig id	Seq. length	%GC	circularity	Plasmid database accession	Host taxonomy
2	5597	56.9	circular	-	-

155

156 **Table 7.** Potential phage sequences identified in the test strain and their association with genes of concern.

Contig ID	Start (bp)	Stop (bp)	Association to genes of concern
1	1,870,815	1,874,260	No

157

158 3.4. Taxonomic identification

159 3.4.1. Preliminary identification and selection of the strains

160 Taxonomic identification of a bacterial strain usually starts with the 16S ribosomal RNA (bacterial small
161 subunit, SSU, rRNA) gene. The analysis was based on a blast-based search against a curated database (**Table**

162 8). The closest type strain and complete 16S ribosomal RNA sequence match was *Corynebacterium*
163 *glutamicum* ATCC 13032.

164 **Table 8.** 16S rRNA analysis. The total score is the sum of alignment scores of all segments from the same
165 database sequence that match the query sequence (calculated over all segments). Query coverage shows
166 the % of the contig that aligns with the NCBI hit. Percent identity is the similarity of the query sequence
167 to the target sequence. The results were sorted by their percent identity due to the prevalence of partial
168 sequences in the results.

Sequence Name	Accession	Identity (%)	Query Coverage (%)	Total Score
Corynebacterium glutamicum strain ATCC 13032 16S ribosomal RNA, partial sequence	NR_041817.1	100.00	100 %	2809
Corynebacterium glutamicum strain ATCC 13032 16S ribosomal RNA, partial sequence	NR_074663.1	99.87	100 %	2798
Corynebacterium suranareeae strain N24 16S ribosomal RNA, partial sequence	NR_180402.1	98.70	95 %	2584
Corynebacterium crudilactis strain JZ16 16S ribosomal RNA, partial sequence	NR_153680.1	98.27	94 %	2527
Corynebacterium deserti GIMN1.010 16S ribosomal RNA, partial sequence	NR_118005.1	97.92	97 %	2566
Corynebacterium callunae strain NCIMB 10338 16S ribosomal RNA, partial sequence	NR_037036.1	97.13	98 %	2521
Corynebacterium callunae strain CCUG 28793 16S ribosomal RNA, partial sequence	NR_119132.1	96.53	86 %	2196
Corynebacterium pacaense strain Marseille-P2417 16S ribosomal RNA, partial sequence	NR_179516.1	96.51	97 %	2453
Corynebacterium faecale strain YIM 101505 16S ribosomal RNA, partial sequence	NR_149764.1	95.98	99 %	2459
Corynebacterium efficiens YS-314 16S ribosomal RNA, partial sequence	NR_102865.1	95.79	99 %	2435
Corynebacterium occultum strain 2039 16S ribosomal RNA, partial sequence	NR_180960.1	95.45	99 %	2403
Corynebacterium pollutisoli strain VDS11 16S ribosomal RNA, partial sequence	NR_151947.1	95.40	90 %	2176
Corynebacterium efficiens YS-314 16S ribosomal RNA, partial sequence	NR_024772.1	95.36	95 %	2314
Corynebacterium epidermidicanis strain 410 16S ribosomal RNA, partial sequence	NR_108533.1	95.16	94 %	2272
Corynebacterium casei LMG S-19264 16S ribosomal RNA, partial sequence	NR_121719.2	95.10	100 %	2392
Corynebacterium casei LMG S-19264 16S ribosomal RNA, partial sequence	NR_122062.1	95.09	99 %	2386
Corynebacterium kalinowskii strain 1959 16S ribosomal RNA, partial sequence	NR_180958.1	95.00	99 %	2364
Corynebacterium casei LMG S-19264 16S ribosomal RNA, partial sequence	NR_122052.1	94.99	99 %	2364
Corynebacterium hindlerae strain NML 93-0612 16S ribosomal RNA, partial sequence	NR_178402.1	94.93	93 %	2217
Corynebacterium nasicanis strain 2673/12 16S ribosomal RNA, partial sequence	NR_145583.1	94.83	91 %	2158

169

170 Alignment-free genome distance estimation against the NCBI RefSeq representative genome database was
171 used to estimate the global mutation distances. The search showed the best hit (low distance and high
172 matching) to *Corynebacterium glutamicum* ATCC 13032 (GCF_000196335.1) (**Table 9**).

173 **Table 9.** Taxonomic identification of the test strain using alignment-free genome distance estimation.
174 Search was made for the closest matching NCBI RefSeq genomes. The type strains are marked with (T).
175 Inconclusive assembly is marked with (I).

Reference ID	Distance	p-value	Matches	Assembly accession
<i>Corynebacterium glutamicum</i> ATCC 13032 (T)	0.0	0.0	400/400	GCF_000196335.1
<i>Corynebacterium glutamicum</i> K051	0.000315	0.0	396/400	GCF_000382905.1
<i>Corynebacterium glutamicum</i> MB001	0.001539	0.0	381/400	GCF_000445015.1
<i>Corynebacterium glutamicum</i> AR1	0.011105	0.0	288/400	GCF_000742735.1
<i>Corynebacterium glutamicum</i> ATCC 21831	0.011486	0.0	285/400	GCF_000742715.1
<i>Corynebacterium glutamicum</i> B253	0.015341	0.0	257/400	GCF_000828015.1
<i>Corynebacterium glutamicum</i> ATCC 14067	0.017337	0.0	244/400	GCF_002243555.1
<i>Corynebacterium glutamicum</i> SYPS-062-33a	0.017496	0.0	243/400	GCF_000934815.1
<i>Corynebacterium glutamicum</i> SYPS-062	0.017496	0.0	243/400	GCF_000819915.1
<i>Corynebacterium glutamicum</i> R	0.022365	0.0	215/400	GCF_000010225.1
<i>Corynebacterium glutamicum</i> SCgG2	0.022554	0.0	214/400	GCF_000404185.1
<i>Corynebacterium glutamicum</i> SCgG1	0.022554	0.0	214/400	GCF_000404145.1
<i>Corynebacterium crenatum</i> SYPA 5-5 (I)	0.022745	0.0	213/400	GCF_000732145.1
<i>Corynebacterium glutamicum</i> S9114	0.022745	0.0	213/400	GCF_000224315.1
<i>Corynebacterium glutamicum</i> Z188	0.022745	0.0	213/400	GCF_000417765.1
<i>Corynebacterium crenatum</i> MT (I)	0.023131	0.0	211/400	GCF_000380545.1
<i>Corynebacterium callunae</i> DSM 20147 (T)	0.177686	1.034e-19	12/400	GCF_000344785.1
<i>Corynebacterium callunae</i> DSM 20147 (T)	0.182972	9.867e-18	11/400	GCF_000420585.1
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i> NA18	0.210610	7.896e-10	7/400	GCF_000511585.1

176

177 The results from the 16S rRNA analysis and alignment-free genome distance estimation were used for the
178 selection of the *Corynebacterium* strains for the ANI and core genome alignment analyses (**Table 10**).

179 **Table 10.** Sequences included in the phylogenetic analyses. Type strains are marked with (T).

Strain	Assembly Accession	Contigs	Size (bp)	GC%
<i>Corynebacterium glutamicum</i> ATCC 13869	GCF_001687645.1	2	3,300,947	54.3
<i>Corynebacterium glutamicum</i> ATCC 13032 (T)	GCF_000196335.1	1	3,282,708	53.8
<i>Corynebacterium glutamicum</i> K051	GCF_000382905.1	1	3,309,400	53.8
<i>Corynebacterium glutamicum</i> MB001	GCF_000445015.1	1	3,079,253	54.2
<i>Corynebacterium glutamicum</i> AR1	GCF_000742735.1	2	3,162,487	54.1
<i>Corynebacterium glutamicum</i> ATCC 21831	GCF_000742715.1	2	3,192,886	54.1
<i>Corynebacterium glutamicum</i> B253	GCF_000828015.1	2	3,229,314	54.3

Corynebacterium crudilactis JZ16	GCF_001643015.1	3	3,217,288	51.7
Corynebacterium deserti GIMN1.010 (T)	GCF_001277995.1	3	3,033,893	55.3
Corynebacterium callunae DSM 20147 (T)	GCF_000344785.1	3	2,928,683	52.5
Test strain	-	2	3,282,540	53.8

180

181

3.4.2. Alignment-based calculation of average nucleotide identity (ANI)

182

Results from the comparison of the test strain with other *Corynebacterium* strains are shown in **Table 11**.

183

The comparisons indicate that the test strain shares 99.9887% identity with *Corynebacterium glutamicum*

184

strain K051, 99.9857% identity with *Corynebacterium glutamicum* strain MB001, and 99.9835% identity with

185

Corynebacterium glutamicum type strain ATCC 13032 (parental strain). The other type strain genomes of

186

Corynebacterium genus showed identities lower than 95% to the test strain. The cut-off value for species

187

identification is set as 95 %.

188

Table 11. ANI (%) calculations between the test strain and selected *Corynebacterium* strains. Type strains

189

are marked with (T). Closest match is marked in red.

Strain	The Test Strain
<i>C. glutamicum</i> K051	99.9887
<i>C. glutamicum</i> MB001	99.9857
<i>C. glutamicum</i> ATCC 13032 Parental strain (T)	99.9835
<i>C. glutamicum</i> ATCC 21831	98.5908
<i>C. glutamicum</i> AR1	98.549
<i>C. glutamicum</i> ATCC 13869	98.3508
<i>C. glutamicum</i> B253	98.0811
<i>C. crudilactis</i> JZ16 (T)	82.2132
<i>C. deserti</i> GIMN1.010 (T)	80.2336
<i>C. callunae</i> DSM 20147 (T)	76.6464

190

3.4.3. Phylogenetic tree

191

A phylogenetic tree was constructed to confirm the taxonomy of the test strain. The analysis (**Figure 2**) was

192

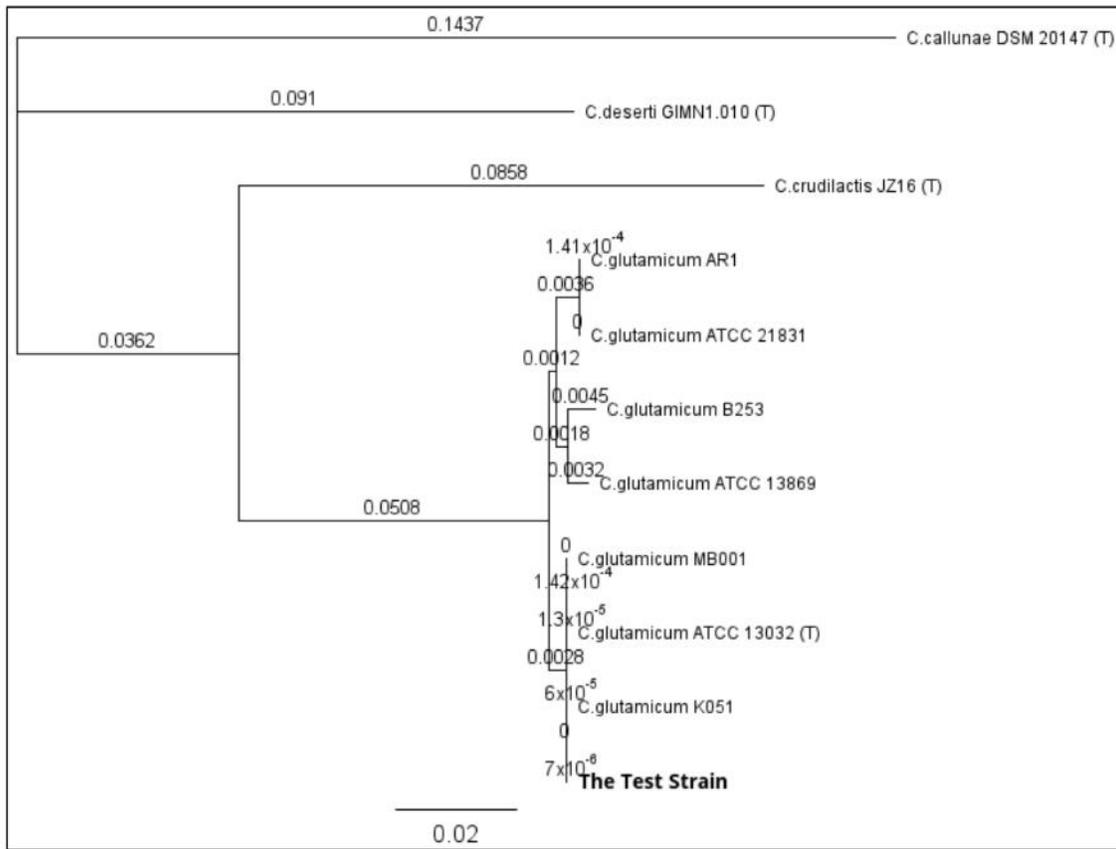
made based on a core genome alignment of 161 genes shared among the selected strains (**Table 10**). The

193

test strain clustered with the *Corynebacterium glutamicum* ATCC 13032, *Corynebacterium glutamicum* MB001

194

and *Corynebacterium* K051.



195

196 **Figure 2.** Phylogenetic tree based on the alignment of 161 core genes among the test strain and the
 197 selected *Corynebacterium* genomes (**Table 10**). The tree was rooted to *Corynebacterium callunae* DSM 21047
 198 type strain. Type strains are marked with (T).

199 **3.4.4. Conclusion about taxonomic identification**

200 Based on the evidence presented above, it is concluded that the test strain is a member of the species
 201 *Corynebacterium glutamicum* and is closely related to *Corynebacterium glutamicum* MB001, *Corynebacterium*
 202 *glutamicum* ATCC 13032 type strain, and *Corynebacterium glutamicum* K051.

203 **3.5. Assessment of potential antimicrobial resistance**

204 Microorganisms should not add to the pool of antimicrobial resistance (AMR) genes and increase the spread
 205 of AMR. A particular attention is paid to antimicrobials relevant for their use in humans and animals, listed
 206 by WHO (World Health Organization, 2019) as critically or highly important antimicrobials (CIA, HIA,
 207 respectively). Intrinsic AMR is not considered as a safety concern. However, when a strain of a typically
 208 susceptible species is resistant to a given antimicrobial, it is considered as “acquired resistance” and
 209 requires further investigation.

210 Screening of the test strain genome against two antimicrobial resistance gene databases resulted in two
211 genes exceeding both the 80% identity and 70% coverage thresholds (EFSA, 2021). Search hits are shown
212 in **Table 13**.

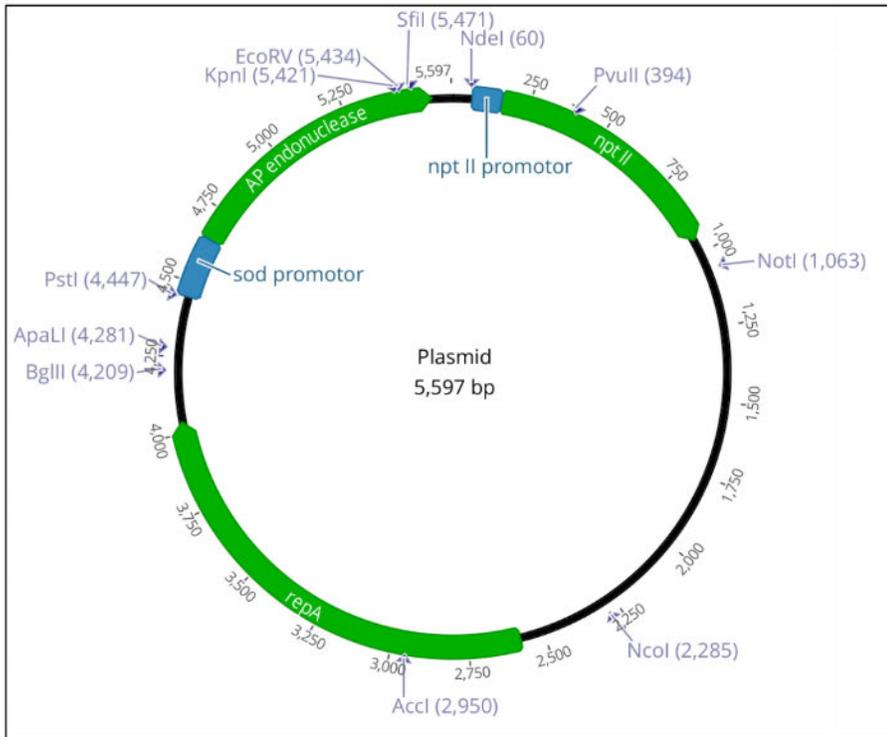
213 **Table 13.** Putative antimicrobial resistance genes in the test strain. The values exceeding the thresholds
214 (EFSA, 2021) are bolded and sorted to the top of the table.

Query Sequence	Query Protein ID	Subject Sequence Name	Subject Sequence Accession	% Coverage	% Identity	Strand	Query Sequence Start	Query Sequence Stop
1	OPFJDDML_02_806	cmr	U43535	100	100	+	2934482	2935861
2	OPFJDDML_03_127	aminoglycoside O-phosphotransferase APH(3')-IIa <i>npt II</i>	WP_000572405.1	100	100	-	3495	4289
1	OPFJDDML_00_160	type B chloramphenicol O-acetyltransferase	WP_031281281.1	25.71	48.15	-	156145	156822
1	OPFJDDML_00_872	trimethoprim-resistant dihydrofolate reductase DfrA18	WP_001031075.1	37.50	50.70	-	897989	898447
1	OPFJDDML_01_562	tetracycline efflux ABC transporter TetAB subunit B	WP_011116970.1	40.72	62.33	-	1607326	1609125
1	OPFJDDML_02_816	NAD(+)-rifampin ADP-ribosyltransferase	WP_044059783.1	39.33	45.76	+	2947101	2947343
1	NA	NAD(+)-rifampin ADP-ribosyltransferase Arr-2b	WP_063842202.1	43.36	54.84	+	2947328	2947513

215 The gene *cmr* exceeding the EFSA threshold is coding for a multidrug efflux MFS transporter protein of
216 *Corynebacterium glutamicum* and is intrinsic and not associated with mobile genetic elements. The gene *npt*
217 *II* encodes aminoglycoside 3'-phosphotransferase and inactivates aminoglycoside antibiotics like
218 kanamycin and was inserted as a selectable marker into the plasmid. The MIC-values for ampicillin,
219 gentamicin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin
220 against *C. glutamicum* KCCM 80101 were equal or below the EFSA MIC cut-off values, indicating that the
221 strain is susceptible to these antimicrobials. The MIC value for kanamycin exceeded eight-fold the EFSA
222 cut-off values for *Corynebacterium* and other Gram-positive bacteria (Biosafe report T1493R1502_2022).
223 *Corynebacterium glutamicum* is a QPS recommended species (EFSA BIOHAZ Panel et al., 2022). Detailed
224 analysis is given in **Appendix 1**.

225 3.6. Description of genetic modifications

226 The test strain was genetically modified for the production of allulose. A plasmid containing the
227 modifications was reported by the client and detected by Biosafe (**Figure 3, Table 12**). The sequence and
228 translation in FASTA format of the plasmid is given in **Appendix 2**.



229

230 **Figure 3.** Map of detected plasmid (5,597 bp) in the test strain. Genetic elements are colored with green
 231 and promotor areas with blue.

232

233 **Table 12.** Detected individual genes of plasmid in the test strain.

Genetic element	Location in sequence (bp)	Size (bp)	Function
<i>AP endonuclease</i>	4,648-5,532	885 bp	D-tagatose 3-epimerase CDS, <i>Flavonifractor plautii</i>
<i>npt II</i>	159-953	795 bp	Npt II, kanamycin resistance protein, <i>Escherichia coli</i> K-12 ER3431
<i>repA</i>	2,582-4,039	1,458 bp	Replication protein, <i>Corynebacterium glutamicum</i> (<i>Brevibacterium saccharolyticum</i>), IPR004322
<i>npt II promoter</i>	64-157	200 bp	Promoter, <i>Escherichia coli</i> K-12 ER3431
<i>sod promoter</i>	4,448-4,647	94 bp	Promoter, <i>Corynebacterium glutamicum</i>

234

235 **4. CONCLUSIONS**

236 Based on the evidence presented above, it is concluded that the test strain is a member of *Corynebacterium*
 237 *glutamicum*, closely related to *Corynebacterium glutamicum* strains K051, ATCC 13032 and MB001. The test
 238 strain contains two AMR genes above the EFSA threshold. The *cmr* gene was not associated with mobile

239 genetic elements and is considered intrinsic. The test strain did not show resistance to any antimicrobials
240 on the MIC panel for tested antimicrobials except for kanamycin where the MIC value exceeded eight-fold
241 the EFSA cut-off values for *Corynebacterium* and other Gram-positive bacteria (Biosafe report
242 T1493R1502_2022). Resistance to kanamycin is explained by the *npt II* gene inserted into the plasmid.
243 *Corynebacterium glutamicum* is a QPS qualified species (EFSA BIOHAZ Panel et al., 2022). No genes of
244 concern were identified.

245 5. REFERENCES

246 EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Rychen G, Aquilina G,
247 Azimonti G, Bampidis V, Bastos M de L, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp
248 J, Kolar B, Kouba M, López-Alonso M, López Puente S, Mantovani A, Mayo B, Ramos F, Saarela M,
249 Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R
250 and Galobart J, 2018. Guidance on the characterisation of microorganisms used as feed additives
251 or as production organisms. EFSA Journal, 16(3), 5206. DOI: 10.2903/j.efsa.2018.5206.

252 EFSA BIOHAZ Panel, Koutsoumanis, Konstantinos, Allende, Ana, Alvarez-Ordóñez, Avelino, Bolton, Declan,
253 Bover-Cid, Sara, Chemaly, Marianne, Davies, Robert, De Cesare, Alessandra, Hilbert, Friederike,
254 Lindqvist, Roland, Nauta, Maarten, Peixe, Luisa, Ru, Giuseppe, Simmons, Marion, Skandamis,
255 Panagiotis, Suffredini, Elisabetta, Cocconcelli, Pier Sandro, Fernández Escámez, Pablo Salvador,
256 Maradona, Miguel Prieto, Querol, Amparo, Sijtsma, Lolke, Suarez, Juan Evaristo, Sundh, Ingvar,
257 Vlak, Just, Barizzone, Fulvio, Hempen, Michaela and Herman, Lieve, 2022. Updated list of QPS-
258 recommended biological agents for safety risk assessments carried out by EFSA. , 6377. DOI:
259 10.5281/ZENODO.1146566.

260 European Food Safety Authority (EFSA), 2021. EFSA statement on the requirements for whole genome
261 sequence analysis of microorganisms intentionally used in the food chain. EFSA Journal, 19(7).
262 DOI: 10.2903/j.efsa.2021.6506.

263 WHO, 2019. Critically important antimicrobials for human medicine, 6th revision. Geneva: World Health
264 Organization. <https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/>.

Appendix B. MIC Report for *Corynebacterium glutamicum* ATCC 13032



Culture Collection of Antimicrobial Resistant Microbes
01797 /The 1st Science building 425,
Wharang-ro 621, Nowonku, Seoul, Korea
TEL) (+) 82-2-970-7724 / FAX) (+) 82-2-970-5901
E-Mail) enjunior@knrc.or.kr / Web-site) www.ccam.or.kr

Certificate of Analysis

1. **Customer :** Daesang Co., LTD
2. **Testing laboratory :** Culture Collection of Antimicrobial Resistant Microbes
3. **Test Purpose :** Minimal Inhibitory concentrations test of 3 *Corynebacterium* spp.
4. **Date of Request :** 2016. Jan. 22
5. **Date of Report :** 2016. Feb. 26
6. **Responsible Staff:** Eunju Shin, Ph.D. Research Professor
Director of Culture Collection of Antimicrobial Resistant Microbes,
(01797) The 1st Science building 425
Wharang-ro 621, Nowon-ku, Seoul, Korea
TEL) 822-970-7724, E-mail) enjunio@knrc.or.kr

Signature

/ Date

Feb. 26, 2016

Certificate of Analysis

1. Purpose of experiment

Assay minimal inhibitory concentrations (MICs) of ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline and vancomycin against *Corynebacterium glutamicum* ATCC13032, *Corynebacterium glutamicum* KCTC 12307BP and *Corynebacterium glutamicum* KFCC 10065.

2. Date of experiment

2016. February. 2. – 2016. February. 24.

3. Method

3.1. Method

The broth dilution method, in accordance with the recommendation of the Clinical Laboratory Standards Institute (CLSI, 2006; 2014), was used to determine the MICs of antimicrobials ampicillin, chloramphenicol (Fluka, St. Louis, Mo, USA), clindamycin, erythromycin, gentamicin, kanamycin, streptomycin (United States Biochemical, OH, USA), tetracycline, and vancomycin. All of the chemicals used in this test were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated. Properly diluted Muller Hinton II (BBL, Sparks, MD, USA) broth containing 5% lysed horse blood and each antimicrobial agent was filled in each well in a 96 well plate. An aliquot containing each isolate was inoculated on each well containing various concentrations of each antimicrobial agent ranging from 0.03 to 128 µg/ml. The final concentration of bacteria was approximately 5×10^4 CFU/well. Then plates were incubated at 35°C for 24 h under ambient condition. MIC is the lowest concentration of antimicrobial agent that completely inhibits the growth of an organism in a well as detected with an unaided eye. *Streptococcus pneumoniae* ATCC 49619 and *Escherichia coli* ATCC 25922 were included in each assay. MIC breakpoint was determined following the criteria of *Corynebacterium* and *Enterobacteriaceae* by CLSI.

3.2. Test Strains

Test strains are shown in Table 1.

Table 1. List of bacterial strains in this report

No.	Name	Strain name	Characteristics
1	<i>Corynebacterium glutamicum</i>	ATCC 13032	Test strain
2	<i>Corynebacterium glutamicum</i>	KCTC 12307BP	Test strain
3	<i>Corynebacterium glutamicum</i>	KFCC 10065	Test strain
4	<i>Streptococcus pneumoniae</i>	ATCC 49619	Quality control
5	<i>Escherichia coli</i>	ATCC 25922	Quality control

4. Result

MICs of 9 antimicrobial agents are shown in Table 2.

Table 2. MICs of 3 strains of *C. glutamicum* against 9 antimicrobial agents ($\mu\text{g/ml}$)

Antimicrobial	Strain	<i>C. glutamicum</i>	<i>C. glutamicum</i>	<i>C. glutamicum</i>	<i>S. pneumoniae</i>	<i>E. coli</i>	MIC ^a	MCV ^b	
	ATCC 13032	KCTC 12307BP	KFCC 10065	ATCC 49619	ATCC 25922	S \leq		R $>$	
ampicillin	0.25	0.06	0.12	0.06	-	-	32 ^d	1	1
chloramphenicol	2	2	2	4	-	-	32 ^d	2	2
clindamycin	2	2	2	0.06	-	4 ^c		0.25	0.25
erythromycin	0.25	0.06	0.06	0.06	-	2 ^c		0.5	0.5
gentamicin	≤ 0.03	≤ 0.03	≤ 0.03	-	0.5	16 ^c		4	4
kanamycin	0.06	0.03	0.03	-	4	-	64 ^d	16	16
streptomycin	0.5	0.25	128	-	4	-	-	8	8
tetracycline	0.5	0.25	0.5	0.25	-	16 ^c		2	2
vancomycin	0.5	0.5	0.5	0.25	-	4 ^c		2	2

^a, minimal inhibitory concentration; ^b, European Food Safety Authority (EFSA) microbiological cut-off values for other Gram+ bacteria

^c, Criteria limit for *Corynebacterium* resistance according to the Clinical Laboratory Standards Institute; ^d, Criteria limit for Enterobacteriaceae resistance according to Clinical Laboratory Standards Institute

5. Discussion

MICs for *C. glutamicum* ATCC 13032, *C. glutamicum* KCTC 12307BP and *C. glutamicum* KFCC 10065 ranged from ≤ 0.03 –2 $\mu\text{g/ml}$ for 9 antimicrobial agents except streptomycin MIC (>128 $\mu\text{g/ml}$) for *C. glutamicum* KFCC 10065. The MICs of clindamycin were higher than the limit for susceptible criteria.

6. Reference

Clinical and Laboratory Standards Institute 2014. Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement M100-S24, Wayne, Pa.

Clinical and Laboratory Standards Institute 2006. Method for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious Bacteria; Approved guideline M45-A, Wayne, Pa.

European Committee on Antimicrobial Susceptibility Testing. Antimicrobial wild type distributions of microorganisms, EUCAST version 5.13. Available at [www. Eucast. Org](http://www.Eucast.Org) (Online, accessed Feb, 22, 2016)

European Food Safety Authority (EFSA) 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal*. 10, 2740. <http://www.efsa.europa.eu/efsajournal>, doi:10.2903/j.efsa.2012.2740.

Culture Collection of Antimicrobial Resistant Microbes



Appendix C. MIC Values of *Corynebacterium glutamicum* KCCM 80101 or DS00001



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REPORT T1493R1502/2022
11.1.2023
CONFIDENTIAL

STUDY REPORT

Determination of the minimum inhibitory concentration of selected antimicrobials against *Corynebacterium glutamicum* KCCM 80101

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EXECUTIVE SUMMARY

Scope: The objective was to analyse the minimum inhibitory concentration (MIC) of ampicillin, gentamicin, kanamycin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin against *Corynebacterium glutamicum* KCCM 80101 (hereafter *C. glutamicum* KCCM 80101).

Strategy: The MICs were assessed with accredited broth microdilution method according to CLSI standard M07, 11th ed. 'Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically' and CLSI standard M45, 3rd ed. 'Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria'. The antimicrobial susceptibility test was performed in triplicate in custom-made Sensititre™ FINBIOS1 96-well plates under aerobic conditions at $+35 \pm 2$ °C for $24\text{h} \pm 2$ h using cation-adjusted Mueller Hinton Broth supplemented with 5% lysed horse blood. Due to susceptible test result for ampicillin, the incubation was extended according to CLSI M45 standard for additional 24 h and results for ampicillin were recorded after 48 h incubation. *Streptococcus pneumoniae* DSM 24048 (ATCC 49619) and *Escherichia coli* ATCC 25922 were used as quality control strains.

Results: The MIC values against the quality control strains were all within the acceptable limits. The MIC-values for ampicillin, gentamicin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin against *C. glutamicum* KCCM 80101 were equal or below the EFSA MIC cut-off values. The MIC value for kanamycin exceeded the EFSA MIC cut-off value for *Corynebacterium* and other Gram-positive with three dilution steps (8x).

Conclusion: The MIC-values for ampicillin, gentamicin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin against *C. glutamicum* KCCM 80101 were equal or below the EFSA MIC cut-off values, indicating that the strain is susceptible to these antimicrobials. The MIC value for kanamycin exceeded eight-fold the EFSA cut-off values for *Corynebacterium* and other Gram-positive and hence the molecular basis for kanamycin tolerance should be elucidated.

1 INTRODUCTION

Biosafe Ltd was requested to provide an analysis of the minimum inhibitory concentrations (MICs) for ampicillin, gentamicin, kanamycin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin against *Corynebacterium glutamicum* KCCM 80101 (hereafter *C. glutamicum* KCCM 80101).

2 TEST MATERIAL, DATA AND METHODS

2.1 Test material

The test material and essential dates related to notification of the study according to transparency regulation (EU 2019/1381) are described in **Table 1**.

Table 1. Date of the test material arrival for antimicrobial susceptibility analysis, notified starting and completion date of the study and date of analysis.

Test material (<i>C. glutamicum</i> KCCM 80101, freeze-dried, provided by Daesang Corp.) arrival	15.8.2022
Notified starting date of the study	19.9.2022
Analysis date	21.9.2022
Notified completion date of the study	30.11.2022

2.2 Methods

The antimicrobial susceptibility against ampicillin, gentamicin, kanamycin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin was tested with broth microdilution method according to CLSI standard M07 (11th ed., 2018), which describes the general method, except that custom-made microdilution trays were used (see below). CLSI standard M45 (3rd ed., 2016) for *Corynebacterium* spp. was followed in the selection of medium and incubation conditions. The A_{550} was adjusted to 0.125 (± 0.01), which corresponds to approximately 6.9×10^7 cfu/mL based on a preliminary study. The cell suspension was further diluted in broth to reach 5×10^4 cfu/well for the test.

The test was performed in triplicate in custom-made Sensititre™ FINBIOS1 96-well plates (Thermo Scientific) under aerobic conditions at 35 ± 2 °C using cation-adjusted Mueller Hinton Broth (Oxoid, CM0405, cations Mg^{2+} and Ca^{2+} added separately) supplemented with 5% lysed horse blood (Thermo Scientific) for $24h \pm 2$ h. Due to susceptible test result for ampicillin, the incubation was extended according to CLSI M45 standard for additional 24 h and results for ampicillin were recorded after 48 h incubation. *Streptococcus pneumoniae* DSM 24048 (ATCC 49619) used as a quality control strain was incubated for 22 ± 2 h. *Escherichia coli* ATCC 25922 used as a quality control strain for gentamicin was incubated for 18 ± 2 h.



Biosafe Ltd is a Finnish Accreditation Service (FINAS) accredited testing laboratory T356, accreditation requirement SFS-EN ISO/IEC 17025:2017. Antimicrobial susceptibility testing for *Corynebacterium glutamicum* with broth microdilution method using FINBIOS1 microdilution trays falls in the scope of accreditation.

3 RESULTS AND DISCUSSION

The result of the antimicrobial susceptibility test is shown in **Tables 2 to 4**. The replicate data is included in **Appendix 1**. The MIC values for the quality control strains were all within the acceptable limits presented in CLSI standard M100 (32nd ed., 2022) (**Tables 3 and 4**). The MIC values for *C. glutamicum*

KCCM 80101 were compared with the MIC cut-off values reported by EFSA for *Corynebacterium* and other Gram-positive (Table 2). The MIC-values for ampicillin, gentamicin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin against *C. glutamicum* KCCM 80101 were equal or below the EFSA MIC cut-off values. The MIC value for kanamycin exceeded the EFSA MIC cut-off value for *Corynebacterium* and other Gram-positive with three dilution steps (8x).

Table 2. Susceptibility of *C. glutamicum* KCCM 80101 to the antimicrobials required to be tested by EFSA. The values are mode of three replicate analyses (values that differ from the mode are noted in the footnote).

Antimicrobial	MIC (µg/mL)	EFSA MIC cut-off values for <i>Corynebacterium</i> (µg/mL)
Ampicillin	≤0.25 ^a	1
Gentamicin	≤0.12	4
Kanamycin	128 ^b	16
Streptomycin	≤2	8
Tetracycline	0.5 ^c	2
Erythromycin	0.5	1
Clindamycin	2	4
Chloramphenicol	4	4
Vancomycin	≤0.25	4

^a The result for ampicillin was recorded after 48h

^b One of the three replicates presented a MIC of 256 µg/mL

^c One of the three replicates presented a MIC of 1 µg/mL

Table 3. Susceptibility of the quality control strain *Streptococcus pneumoniae* DSM 24048 (ATCC 49619) to antimicrobials required to be tested by EFSA. The MIC Quality Control range for fastidious organisms (Broth Dilution Methods), specifically for *Streptococcus pneumoniae* DSM 24048 (ATCC 49619), according to CLSI standard M100 (32nd ed., 2022, Table 5B) is shown in the right-hand column.

Antimicrobial	MIC (µg/mL)	<i>S. pneumoniae</i> DSM 24048 (ATCC 49619) (µg/mL)
Ampicillin	≤0.25	0.06-0.25
Kanamycin	32	- ^a
Streptomycin	16	- ^a
Tetracycline	0.5	0.06-0.5
Erythromycin	≤0.12	0.03-0.12
Clindamycin	0.06	0.03-0.12
Chloramphenicol	2	2-8
Vancomycin	≤0.25	0.12-0.5

^a The acceptable range of MIC values for kanamycin and streptomycin are not available for the quality control strain *Streptococcus pneumoniae* ATCC 49619.

Table 4. Susceptibility of the quality control strain *Escherichia coli* ATCC 25922 to gentamicin. The range of accepted MIC-values presented in CLSI M45 (3rd ed., 2016, Table 23B) is shown in the right-hand column.

Antimicrobial	MIC (µg/mL)	<i>Escherichia coli</i> ATCC 25922 (µg/mL)
Gentamicin	0.5 ^a	0.25-1

^a One of the three replicates presented a MIC of 1 µg/mL

4 CONCLUSIONS

The MIC-values for ampicillin, gentamicin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol and vancomycin against *C. glutamicum* KCCM 80101 were equal or below the EFSA MIC cut-off values, indicating that the strain is susceptible to these antimicrobials. The MIC value for kanamycin exceeded eight-fold the EFSA cut-off values for *Corynebacterium* and other Gram-positive and hence the molecular basis for kanamycin tolerance should be elucidated.

REFERENCES

CLSI. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. 3rd ed. CLSI guideline M45. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2022.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos ML, Bories G, Chesson A, Cocconcelli PS, Flachowsky G, Gropp J, Kolar B, Kouba M, Lopez-Alonso M, Lopez Puente S, Mantovani A, Mayo B, Ramos F, Saarela M, Villa RE, Wallace RJ, Wester P, Glandorf B, Herman L, Kärenlampi S, Aguilera J, Anguita M, Brozzi R, Galobart J, 2018. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA Journal, 16(3), 5206, 24 pp. <https://doi.org/10.2903/j.efsa.2018.5206>.

APPENDICES

Appendix 1. Antimicrobial susceptibility testing replicate data

Note

The samples will be stored for three months after reporting the results.
 Copies of all the laboratory data are available to the client on request.

APPENDIX 1. Antimicrobial susceptibility testing replicate data

Table 1. MIC values (µg/mL) for three replicates of *C. glutamicum* KCCM 80101

Antimicrobial	Replicate 1	Replicate 2	Replicate 3
Ampicillin	≤0.25	≤0.25	≤0.25
Gentamicin	≤0.12	≤0.12	≤0.12
Kanamycin	128	128	256
Streptomycin	≤2	≤2	≤2
Tetracycline	0.5	0.5	1
Erythromycin	0.5	0.5	0.5
Clindamycin	2	2	2
Chloramphenicol	4	4	4
Vancomycin	≤0.25	≤0.25	≤0.25

Table 2. MIC values (µg/mL) for three replicates of *Streptococcus pneumoniae* DSM 24048 (ATCC 49619)

Antimicrobial	Replicate 1	Replicate 2	Replicate 3
Ampicillin	≤0.25	≤0.25	≤0.25
Kanamycin	32	32	32
Streptomycin	16	16	16
Tetracycline	0.5	0.5	0.5
Erythromycin	≤0.12	≤0.12	≤0.12
Clindamycin	0.06	0.06	0.06
Chloramphenicol	2	2	2
Vancomycin	≤0.25	≤0.25	≤0.25

Table 3. MIC values (µg/mL) for three replicates of *Escherichia coli* ATCC 25922

Antimicrobial	Replicate 1	Replicate 2	Replicate 3
Gentamicin	0.5	0.5	1

Appendix D. Gene Transfer Report



Horizontal Gene Transfer Assay for *Corynebacterium glutamicum* DS00001

Client : Daesang Corporation

Bio-Evaluation Center,
Korea Institute of Bioscience and Biotechnology
2023.04.25.



Statement

Title: Horizontal Gene Transfer Assay for *Corynebacterium glutamicum* DS00001

This safety assessment was performed to test horizontal gene transfer by plasmid conjugation of *Corynebacterium glutamicum* DS00001.


Ju Seok Lee, Ph.D.
Senior Research Scientist

4/27/2023
Date

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Test Overview

Client	Name	Daesang Corporation
	Location	120, Changgyeonggung-ro, Jongno-gu, Seoul, Korea
	Person in Charge	Sanghee Lee
	Contact Information	+82-2-490-5068(☎), +82-2-2657-5277 (fax)
Test Institute	Name	Bio-Evaluation Center, Korea Institute of Bioscience and Biotechnology
	Location	30 Yeongudanji-ro, Ochang-eup, Cheongwon-gu, Cheongju-si, Republic of Korea
	Person in Charge	Ju Seok Lee
	Contact Information	+82-43-240-6526 (☎), +82-43-240-6549 (fax)
시험일정	2022.09.27	Acquisition of samples from Client.
	2022.10.01	Start the horizontal gene transfer assay
	2023.03.20	End the horizontal gene transfer assay
	2023.04.25	Submission of the report

Persons in Charge

Field	Name	Role
Horizontal Gene Transfer Test	Ju Seok Lee	Experiment design and Write a report
Horizontal Gene Transfer Test	Jiyoun Kim	Manage the microorganisms and Conjugation
Horizontal Gene Transfer Test	Yeonju Kwon	Manage the microorganisms and Conjugation

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I. Summary

Purpose of Test

It was performed to evaluate the horizontal gene transfer frequency by conjugation of genetically modified microorganism, *Corynebacterium glutamicum* DS00001, which has recombinant plasmid FDPE (hereinafter referred to as plasmid DNA).

Experimental Approach

The conjugation test was performed by filter-mating method using *Corynebacterium glutamicum* DS00001 as donor, and twenty of related and pathogenic rifampicin resistance-induced microorganisms. To discriminate the transconjugant from donor and recipient, conjugants were grown with double antibiotic selection marker followed by colony selection. To identify the false-positive colony, which is donor acquired the rifampicin resistance by spontaneous mutation, PCR with insert gene- and donor-specific primer were employed. The horizontal gene transfer frequency by the calculation of colony counting of recipient microorganisms showing dual antibiotic resistance.

Result

The gene transfer frequencies by conjugation among twenty tested microorganisms were below the detection limit, 4.06×10^{-10} . These conjugation frequencies are significantly lower than the minimum transfer frequencies of 1.3×10^{-9} derived from literature search. Thus, the horizontal gene transfer by conjugation between donor and twenty of recipient was not identified under current experiment condition.

II. Material and Method

1. Microorganisms

Donors were obtained from client and used for further analysis. All recipients were obtained from Korean collection for type cultures (KCTC, Korea), Korean Culture Center of Microorganisms (KCCM, Korea), Korean Agricultural Culture Collection (KACC, Korea). Detailed information and growth condition for all microorganisms used in this test were described in Table 1. All recipient was treated with 0.2% (v/v) of ethyl methanesulfonate (EMS) to induce rifampicin resistance, and selected under 100 µg/ml of rifampicin condition.

Table 1. Donor and Recipient Information

Category	Name	ID	Growth Condition	
			Medium ¹	Temperature (°C)
Donor				
Donor	<i>Corynebacterium glutamicum</i> DS00001	-	TSB	30
Recipients				
Relatives	<i>Corynebacterium glutamicum</i>	KCTC 9097	TSB	30
	<i>Corynebacterium</i> sp. IC10	KCCM 43306	TSB	30
	<i>Corynebacterium xerosis</i>	KCCM 40941	TSB	37
	<i>Corynebacterium ammoniagenes</i>	KCTC 1018	TSB	30
	<i>Corynebacterium otitidis</i>	KACC 16289	TSB	30
	<i>Corynebacterium nuruki</i>	KACC 15032	NB	37
	<i>Corynebacterium deserti</i>	KACC 16400	NB	37
	<i>Corynebacterium diphtheriae</i>	KCCM 40413	NB	37
Pathogenic	<i>Enterobacter cloacae</i> subsp. <i>cloacae</i>	KCTC 2361	NB	37
	<i>Escherichia coli</i> K-12	KCTC 1116	NB	37
	<i>Escherichia fergusonii</i>	KCTC 22525	NB	37
	<i>Staphylococcus epidermidis</i>	KCCM 35494	TSB	30
	<i>Shigella flexneri</i>	KCTC 22192	TSB	30
	<i>Klebsiella pneumoniae</i>	KCTC 22058	TSB	30
	<i>Shigella sonnei</i> (Levine) Weldin	KCTC 22530	TSB	30
	<i>Tsukamurella inchoensis</i>	KACC 20573	TSB	30
	<i>Gordonia effusa</i>	KACC 16276	TSB	30
	<i>Tsukamurella tyrosinosolvans</i>	KACC 20575	TSB	30
	<i>Dietzia papillomatosis</i>	KACC 20997	TSB	30
	<i>Tsukamurella strandjordii</i>	KACC 20644	TSB	30

¹NB: Nutrient broth (BD Bioscience, USA), TSB: Tryptic soy broth (BD Bioscience, USA).

2. Conjugation Test by Filter-mating Method

To evaluate the conjugation frequency, filter-mating method were employed [1, 2]. 10 ml of donor and recipients were grown at their optimal growth condition for more than 12 hours. Donor and each recipient cell culture were mixed in 1:8 (v/v) ratio, followed by the 3 time washing with 1X phosphate-buffered saline (PBS) to remove remnant antibiotic and medium. The mixture was resuspended with 150 μ l of 1X PBS buffer. The 50 μ l of mixture in 1X PBS buffer were collected on the 25mm membrane filter (pore size 0.2- μ m, ADVANTEC, Korea), which were placed on the Nutrient agar plate without any antibiotic, and incubate for 24 hours at recipient optimal growth temperature. The detailed test condition was described in Table 3. After 24 hours, filter membrane with the mixture was transferred to 50 ml conical tube and harvest the mixture with vortexing for 30 seconds. The conjugation frequency was estimated by the Equation 1. The number of transconjugants, which the recipients having recombinant plasmid FDPE, were calculated by the mixture spreading on the Nutrient agar plates with kanamycin (25 μ g/ml) and rifampicin (100 μ g/ml). The plates were grown in 30°C for 48 hours followed by the colony counting. The number of recipients were calculated by the mixture spreading on the recipient's optimal growth condition with rifampicin antibiotic (100 μ g/ml), since it have been known that the number of transconjugants were extremely small, and could be ignored. To identify the false-positive, donor acquired the rifampicin resistance by spontaneous mutation, PCR with plasmid- and donor-specific primer pair. Detailed information of PCR primer set and results were in Supplementary Information. All tests were replicated in three times.

$$\text{The conjugation frequency} = \frac{\text{The number of transconjugants}}{\text{The number of recipients}} \quad (\text{Eq. 1})$$

III. Results

It is considered that there is no internationally established guideline for safe range of gene transfer frequency. We have searched for literatures regarding the study of gene transfer frequencies and found no case of gene transfer frequencies exceeding 1.3×10^{-9} and used this information for the evaluation of this study (Table 2). The results of plasmid DNA transfer frequencies of *Corynebacterium glutamicum* DS00001 to twenty recipient microorganisms by filter-mating methods are summarized in Table 3. The horizontal gene transfer frequencies by conjugation among twenty tested microorganisms were below the detection limit ($<4.06 \times 10^{-10}$) (Table 3). These rates are equivalent to the minimum transfer frequencies of 1.3×10^{-9} derived from literature search (Table 2). In conclusion, the horizontal gene transfer by conjugation between donor and twenty of recipient was not identified under current experiment condition.

Table 2. Conjugation frequency in various donor and recipients

Donor	Recipient	Plasmid	Conjugation Method	Conjugation Frequency	Reference
<i>L. lactis</i> SH4147	<i>L. lactis</i> subsp. <i>Lactis</i>	pAM β 1	Filter-mating	6.0×10^{-3}	[1]
			Solid-mating	1.4×10^{-3}	
			Liquid-mating	2.3×10^{-7}	
tetracycline-resistant <i>Lactobacillus</i>	<i>E. faecalis</i> JH2-2	Natural	Filter-mating	6.5×10^{-5}	[3]
	lactose-negative		Filter-mating	1.3×10^{-5}	
	<i>L. lactis</i> subsp. <i>lactis</i> BU2-60				
<i>P. putida</i> Cg1	<i>P. putida</i> KT2440-Tc	pCg1	Solid-mating	2.7×10^{-7}	[2]
			Liquid-mating	1.3×10^{-9}	
<i>E. coli</i> HB101	<i>E. faecalis</i> BM4110	pAT187	Filter-mating	$<1.0 \times 10^{-8}$	[4]
	<i>S. aureus</i> 80CR5	pAT187	Filter-mating	$<1.0 \times 10^{-8}$	
<i>S. faecalis</i>	<i>L. plantarum</i>	pAM β 1	Filter-mating	7.4×10^{-8}	[5]

Table 3. Conjugation condition and frequency of *Corynebacterium glutamicum* DS00001 and 20 recipients

Category	Recipients	Test Condition		Conjugation Frequency ²
		Medium ¹	Temperature (°C)	
Relative	<i>Corynebacterium glutamicum</i>	TSB	30	<5.51 x10 ⁻¹²
	<i>Corynebacterium</i> sp. IC10	TSB	30	<4.06 x10 ⁻¹⁰
	<i>Corynebacterium xerosis</i>	TSB	37	<6.32 x10 ⁻¹¹
	<i>Corynebacterium ammoniagenes</i>	TSB	30	<9.12 x10 ⁻¹¹
	<i>Corynebacterium otitidis</i>	TSB	30	<2.23 x10 ⁻¹⁰
	<i>Corynebacterium nuruki</i>	NB	37	<4.86 x10 ⁻¹¹
	<i>Corynebacterium deserti</i>	NB	37	<1.13 x10 ⁻¹⁰
	<i>Corynebacterium diphtheriae</i>	NB	37	<9.38 x10 ⁻¹¹
Pathogenic	<i>Enterobacter cloacae</i> subsp. <i>cloacae</i>	NB	37	<1.72 x10 ⁻¹¹
	<i>Escherichia coli</i> K-12	NB	37	<1.02 x10 ⁻¹¹
	<i>Escherichia fergusonii</i>	NB	37	<9.99 x10 ⁻¹²
	<i>Staphylococcus epidermidis</i>	TSB	30	<2.18 x10 ⁻¹¹
	<i>Shigella flexneri</i>	TSB	30	<1.99 x10 ⁻¹¹
	<i>Klebsiella pneumoniae</i>	TSB	30	<4.78 x10 ⁻¹²
	<i>Shigella sonnei</i> (Levine) Weldin	TSB	30	<4.94 x10 ⁻¹¹
	<i>Tsukamurella inchonensis</i>	TSB	30	<1.32 x10 ⁻¹⁰
	<i>Gordonia effusa</i>	TSB	30	<7.74 x10 ⁻¹¹
	<i>Tsukamurella tyrosinosolvans</i>	TSB	30	<4.20 x10 ⁻¹¹
	<i>Dietzia papillomatosi</i>	TSB	30	<2.89 x10 ⁻¹⁰
	<i>Tsukamurella strandjordii</i>	TSB	30	<7.74 x10 ⁻¹¹

¹NB: Nutrient broth (BD Bioscience, USA), TSB: Tryptic soy broth (BD Bioscience, USA).

²Frequencies are expressed as the number of transconjugants per donor cell

IV. References

1. Lampkowska, J., et al., *A standardized conjugation protocol to assess antibiotic resistance transfer between lactococcal species*. Int J Food Microbiol, 2008. **127**(1-2): p. 172-5.
2. Park, W., et al., *Identification and Characterization of the Conjugal Transfer Region of the pCg1 plasmid from Naphthalene-Degrading Pseudomonas putida Cg1*. Applied and Environmental Microbiology, 2003. **69**(6): p. 3263-3271.
3. Gevers, D., G. Huys, and J. Swings, *In vitro conjugal transfer of tetracycline resistance from Lactobacillus isolates to other Gram-positive bacteria*. FEMS Microbiology Letters, 2003. **225**(1): p. 125-130.
4. Trieu-Cuot, P., et al., *Plasmid transfer by conjugation from Escherichia coli to Gram-positive bacteria*. FEMS Microbiology Letters, 1987. **48**(1-2): p. 289-294.
5. Sasaki, Y., N. Taketomo, and T. Sasaki, *Factors affecting transfer frequency of pAM beta 1 from Streptococcus faecalis to Lactobacillus plantarum*. Journal of Bacteriology, 1988. **170**(12): p. 5939-5942.
6. Koressaar, T., et al., *Primer3_masker: integrating masking of template sequence with primer design software*. Bioinformatics, 2018. **34**(11): p. 1937-1938.

Supplementary Information: False-positive colony identification

Materials and Methods

A few recipients showed colonies in NB agar plate with both antibiotic, kanamycin and rifampicin. To confirm whether these are transconjugants or false-positive due to the spontaneous mutation of *rpoB* gene in donor, colony PCR test with donor genome-specific and plasmid-specific primer set was conducted.

1. Primer Design

Plasmid-specific primer set were derived from the foreign gene sequence information in plasmid FDPE. Donor-specific primer set were derived from the *Corynebacterium glutamicum* reference genome sequence (NC_021352.1). Only unique region of *Corynebacterium glutamicum* were extracted using blastn (version 2.12.0) with comparing other recipient genome sequence information from NCBI database. Primer3 was used to design primers[6]. Primers were purchased from the vendor (Bioneer, Korea). Detailed primer information is in Table S1. Donor genomic DNA includes donor genome and plasmid genome. If PCR was performed using donor genomic DNA as template, the PCR amplified band should be observed in both donor genome-specific primer pair and plasmid-specific primer pair. If PCR was performed using recipient genomic DNA, any PCR amplified band should not be observed neither donor genome-specific primer pair nor plasmid-specific primer pair, since recipient genomic DNA didn't have donor genome region and plasmid sequence. In case of transconjugants, which is recipients having plasmid, only PCR amplified band observed in plasmid-specific primer pair.

2. Colony PCR

Among 20 recipients, 6 recipients showed colonies. All colonies observed after the mixture enumerating on the NB agar plates with double antibiotic selection marker were picked and streaked on the NB agar plates with double antibiotic selection marker for further analysis.

The PCR reaction conditions were 94°C 3min, [94°C 30 sec, 60°C 30 sec, 72°C 30 sec] 35 cycles, 72°C 5min extension, using 10 µl of AccuPower® PCR master mix (Bioneer, Korea), 40 pmole of each of forward and reverse primer in 20 µl of PCR reaction volume. The colony were picked by pipet tips and resuspended in PCR reaction solution with pipetting. Weak bands were performed PCR again, to confirm the band amplification.

Results

Among 20 recipients, 6 microorganisms showed colonies in NB agar plates with kanamycin and rifampicin (Table S2). All other colonies were donor, which acquired the rifampicin resistance by spontaneous mutation

Table S1. Primer information for colony PCR

Primer Information		Sequence (5' → 3')	Expected Length (bp)
Plasmid-specific Primer	F	ACCTATTCCATCGGCCTGGA	402
	R	GCTGTCTCTCTCGATGTTCA	
Donor genome-specific primer	F	CTTGCCGTAACCTTCTGGT	591
	R	AGTGCCGTCTGGAAGGAATG	

Table S2. Colony PCR result

Recipient	Colony ¹	Transconjugants ²	False-Positive ³
<i>Corynebacterium</i> sp. IC10	48	-	48
<i>Corynebacterium ammoniagenes</i>	36	-	36
<i>Corynebacterium nuruki</i>	5	-	5
<i>Staphylococcus epidermidis</i>	42	-	42
<i>Shigella flexneri</i>	34	-	34
<i>Shigella sonnei</i> (Levine) Weldin	11	-	11

¹Number of colonies in NB agar plates with kanamycin and rifampicin, after conjugation

²Transconjugants is the recipients, which acquire the plasmid by conjugation

³The donor, which acquire the rifampicin resistance by spontaneous mutation

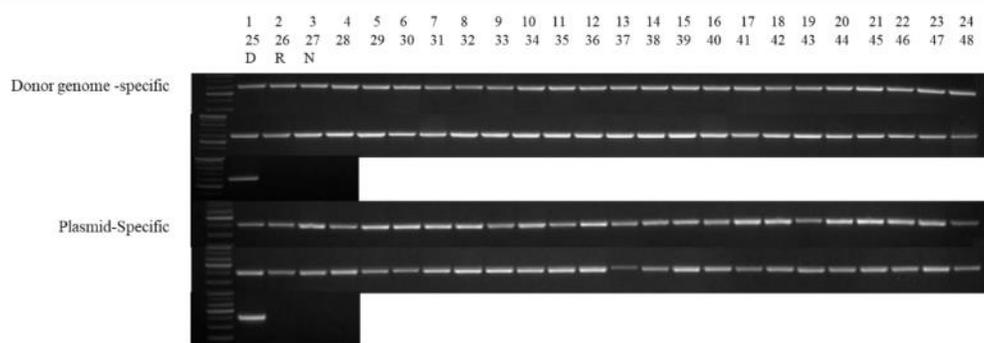


Figure S1. Colony PCR result from *Corynebacterium* sp. IC10 as recipient. PCR with plasmid-specific and donor genome-specific primer were employed. Donor genomic DNA (D), Recipient genomic DNA (R), and negative control (N) are used as control.

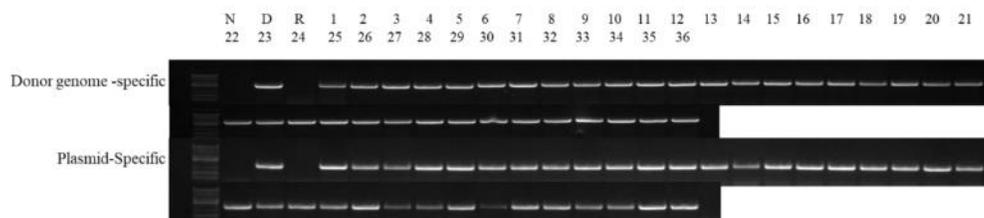


Figure S2. Colony PCR result from *Corynebacterium ammoniagenes* as recipient. PCR with plasmid-specific and donor genome-specific primer were employed. Donor genomic DNA (D), Recipient genomic DNA (R), and negative control (N) are used as control.

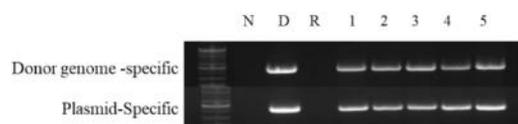


Figure S3. Colony PCR result from *Corynebacterium nuruki* as recipient. PCR with plasmid-specific and donor genome-specific primer were employed. Donor genomic DNA (D), Recipient genomic DNA (R), and negative control (N) are used as control.

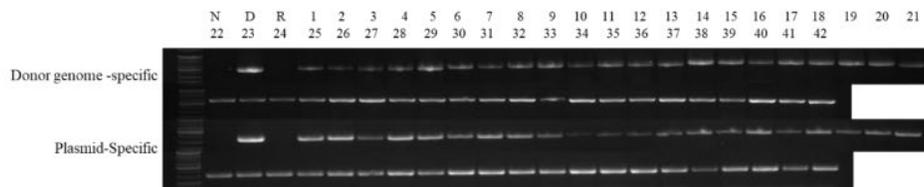


Figure S4. Colony PCR result from *Staphylococcus epidermidis* as recipient. PCR with plasmid-specific and donor genome-specific primer were employed. Donor genomic DNA (D), Recipient genomic DNA (R), and negative control (N) are used as control.

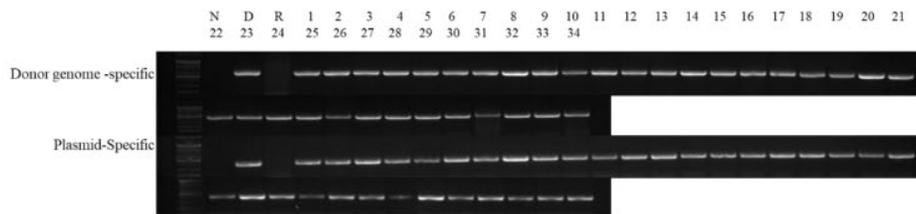


Figure S5. Colony PCR result from *Shigella flexneri* as recipient. PCR with plasmid-specific and donor genome-specific primer were employed. Donor genomic DNA (D), Recipient genomic DNA (R), and negative control (N) are used as control.

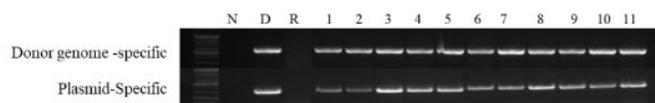


Figure S6. Colony PCR result from *Shigella sonnei (Levine) Weldin* as recipient. PCR with plasmid-specific and donor genome-specific primer were employed.

Appendix E. Revised COAs from Medallion Labs



Medallion Labs

Medallion Order 2022-009808

LOD/LOQ List

Allulose content LOQ: 0.1%

Brix LOD: Dissolved Solids in the range of 0 to ~90%.

Ash LOQ: 0.012% (w/w)

Pb LOQ: 10 ng/g (ppb)

As LOQ: 10 ng/g (ppb)

Cd LOQ: 10 ng/g (ppb)

Fat LOQ: 0.01%

Hg LOQ: 4.00 ppb

Moisture LOQ: 0.012% (w/w)

pH LOD: 0-14

Protein LOD: 0.1%

Sugars LOQ: 0.1%

Aerobic Plate Count LOD: <10 CFU/g

Coliforms LOD: <10CFU/g

E. coli LOD: <10 CFU/g

Molds LOD: <10 CFU/g

Salmonella LOD: 25g

Staphylococcus aureus LOD: <10 CFU/g

Yeast LOD: <10 CFU/g