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

RECEIVED
FEB 2 2017
OFFICE OF
FOOD ADDITIVE SAFETY

On behalf of SamYang Corp.

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
Tel: 410-531-3336
Susanscho1@yahoo.com

GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-Allulose (D-psicose) AS A FOOD INGREDIENT

Table of contents

Tables

Figures

PART 1. SIGNED STATEMENTS AND A CERTIFICATION

Pursuant to 21 C.F.R. Part 170, subpart E, SamYang Corp. submits a Generally Recognized as Safe (GRAS) notice and claims that the use of D-allulose 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.A. Name and Address of the Notifier

Contact person: Dr. Chong-Jin Park
Company name: SamYang Corp.
Address: 295 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, Korea
Telephone number : +82-2-740-7111
E Mail Address: Chongjin.park@samyang.com

1.B. Common or Trade Name

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

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

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

Intended use and use levels of Samyang Corp.'s D-allulose have been adopted from GRN 498 and GRN 400. SamYang Corp. proposes to use D-allulose as a sugar substitute in selected low calorie, reduced calorie, or sugar-free foods including bakery products; beverages; cereals; chewing gums; confections and frostings; frozen dairy desserts; yogurt and frozen yogurt; dressings for salads; gelatins, pudding and fillings; hard and soft candies; jams and jellies; sugar; sugar substitutes; sweet sauces and syrups and fat based creams. Please note that Samyang Corp. has adopted the intended use and use levels mostly from GRN 498 and have added the food categories which are not included in GRN 498, but in GRN 400. Samyang Corp. does not intend to use D-allulose as a component of infant formula or in foods under the USDA's jurisdiction such as meat, poultry, and egg products.

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

As shown in Table 1, SamYang Corp. proposes to use D-allulose as a sugar substitute in food applications at use levels ranging from 2 to 100%.

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

Food category	Maximum use levels, % (w/w)
Bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics)	10-100
Beverages (non-alcoholic), low calorie, reduced calorie, sugar-free	3.5
Cereals, regular Cereals, low calorie, reduced calorie, sugar-free	2 5
Chewing gum	50
Confections and frostings	5
Frozen dairy desserts (ice cream, soft serve, sorbet), low calorie, reduced calorie, sugar-free	5

Yogurt and frozen yogurt, low calorie, reduced calorie, sugar-free	5
Dressings for salads	5
Gelatins, pudding and fillings, low calorie, reduced calorie, sugar-free	10
Hard Candies, low calorie, reduced calorie, sugar-free	50
Soft Candies, low calorie, reduced calorie, sugar-free	25
Jams and jellies	10
Sugar	10
Sugar substitutes	100
Sweet sauces and syrups, low calorie, reduced calorie, sugar-free	10
Fat based cream (used in modified fat/calorie cookies, cakes, pastries, and pie)	5

1.C.3. Purpose for Which the Substance is Used
The substance will be used as a sugar substitute.

1.C.4. Description of the Population Expected to Consume the Substance
The population expected to consume the substance consists of members of general population who consume at least one of the products described above.

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

1.E. 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 NutraSource, Inc.

1.F. 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.G. Certification
We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, available and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

(b) (6)

(b) (6)

Name; Chong-Jin Park, Ph.D. Date

Title: Team leader

Please address correspondence to
Susan S. Cho, Ph.D.
NutraSource, Inc., 6309 Morning Dew Ct, Clarksville, MD 21029
Susanscho1@yahoo.com; 410-531-3336 (O); 301-875-6454 (MP)

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

A. Scientific Information About the Identity of a Notified Substance

2.A.1. Scientific Information Sufficient To Identify a Biological Source

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

Standards of Identity

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

Chemistry, Physicochemical Properties, and Structure

Chemical name is D-ribo-2-ketohexose
MW=180.16
Molecular formula: $C_6H_{12}O_6$
CAS Registry ID; 551-68-8

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

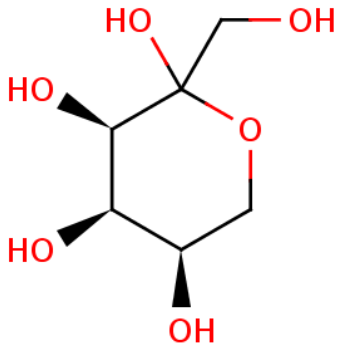


Figure 1. Chemical Structure of D-allulose

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

No toxicant production is expected in the manufacture of allulose. The final product is highly purified through several steps during production. Further, the enzymatic conversion of D-fructose to D-allulose is an enzymatic reaction that occurs in nature, with no known toxicant production.

2.A.3. Particle Size

NLT 90% pass 40 mesh.

2.B. Method of Manufacture

D-allulose is manufactured from fructose in aqueous solution by enzymatic epimerization in the presence of magnesium chloride. The enzyme used is an immobilized D-allulose-3-epimerase, which converts fructose to D-allulose. Compared to those described in previous GRAS notices, SamYang Corp. employs a unique immobilized enzyme system described below. The enzyme system has been proven safe.

Differences in enzyme systems described in various GRNs

Current notice - SamYang Corp.

The neutralized fructose syrup is passed into an immobilized cell system (calcium alginate gel bead with recombinant *Corynebacterium glutamicum* [non-viable cell] harboring D-allulose 3-epimerase [DPE] from *Clostridium scindens*). The fructose then is converted to D-allulose at 50℃.

GRN 400 - CJ CheilJedang

An immobilized cell system (calcium alginate gel bead with *Corynebacterium glutamicum* [non-viable cell] harboring D-psicose 3-epimerase [DPE] originated from *Agrobacterium tumefaciens).*

GRN 498 - Matsutani

D-psicose 3-epimerase (DPE) is extracted from *Escherichia coli* (K12) [non-viable cell] or *Streptomyces violaceoruber* harboring DPE that originated from *Arthrobacter globiformis or Arthrobacter globiformis* itself.

SamYang's Manufacturing process

1. The fructose syrup (≥75% solids concentration) is diluted with clean water (>50% solids concentration) in a reception tank and then stored in a stock tank.
2. The neutralized fructose syrup is passed into an immobilized cell system (calcium alginate gel bead with recombinant *Corynebacterium glutamicum* [non-viable cell] harboring D-allulose 3-epimerase [DPE] from *Clostridium scindens*). The fructose then is converted to D-allulose at 50℃.
3. For decolorization and desalting, the D-allulose solution is mixed with active carbon in a stirred tank reactor. The liquid undergoes pressure filtration to clarify it, and it is treated through an ion exchange process (i.e., a cation column with strongly acidic cationic exchange resin; an anion column with intermediate basic anion exchange resin; and a mixed bed column that has a combination of both strongly acidic and strongly basic resins) to remove any impurities (e.g. calcium, manganese, chloride, and other ionic components, including amino acids, peptides, and proteins).
4. Following ion exchange purification, the D-allulose solution is concentrated with an evaporator to produce syrup (Product 1-Allulose syrup, ≥20% on a dry weight basis).
5. This concentrated syrup is pumped into a separation chromatography system to separate D-allulose from other sugars (i.e., fructose).
6. Using an evaporator, the solution is concentrated to the final density of ≥65 ºBx to produce syrup (Product 2 or 3- D-allulose syrup, ≥50% or ≥90% on a dry weight basis).

7. The final concentrated product is pumped into a batch continuous crystallizer.
8. The crystalline D-allulose (Product 4 - ≥98% D-allulose) is separated by basket centrifugation, washed by spraying distilled water, and finally dried in a rotary dryer.

Quality assurance procedure:

Samyang Corp.'s D-allulose is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. Samyang Corp. 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 grades. D-allulose is manufactured under cGMP using common food industry materials and processes in accordance with the applicable parts of 21 CFR, part 110 of the Code of Federal Regulations. Process tanks and lines are cleaned with sodium hydroxide and hydrogen peroxide following standard procedures common to the dairy industry. 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 2.

Safety of enzymes:

The enzyme utilized is non-toxicological and non-pathogenic. An acute toxicity study showed that a single dose of 2 g/kg bw did not cause any treatment-related abnormalities in Sprague-Dawley rats. The LD_{50} was determined to be far above 2 g/kg bw.

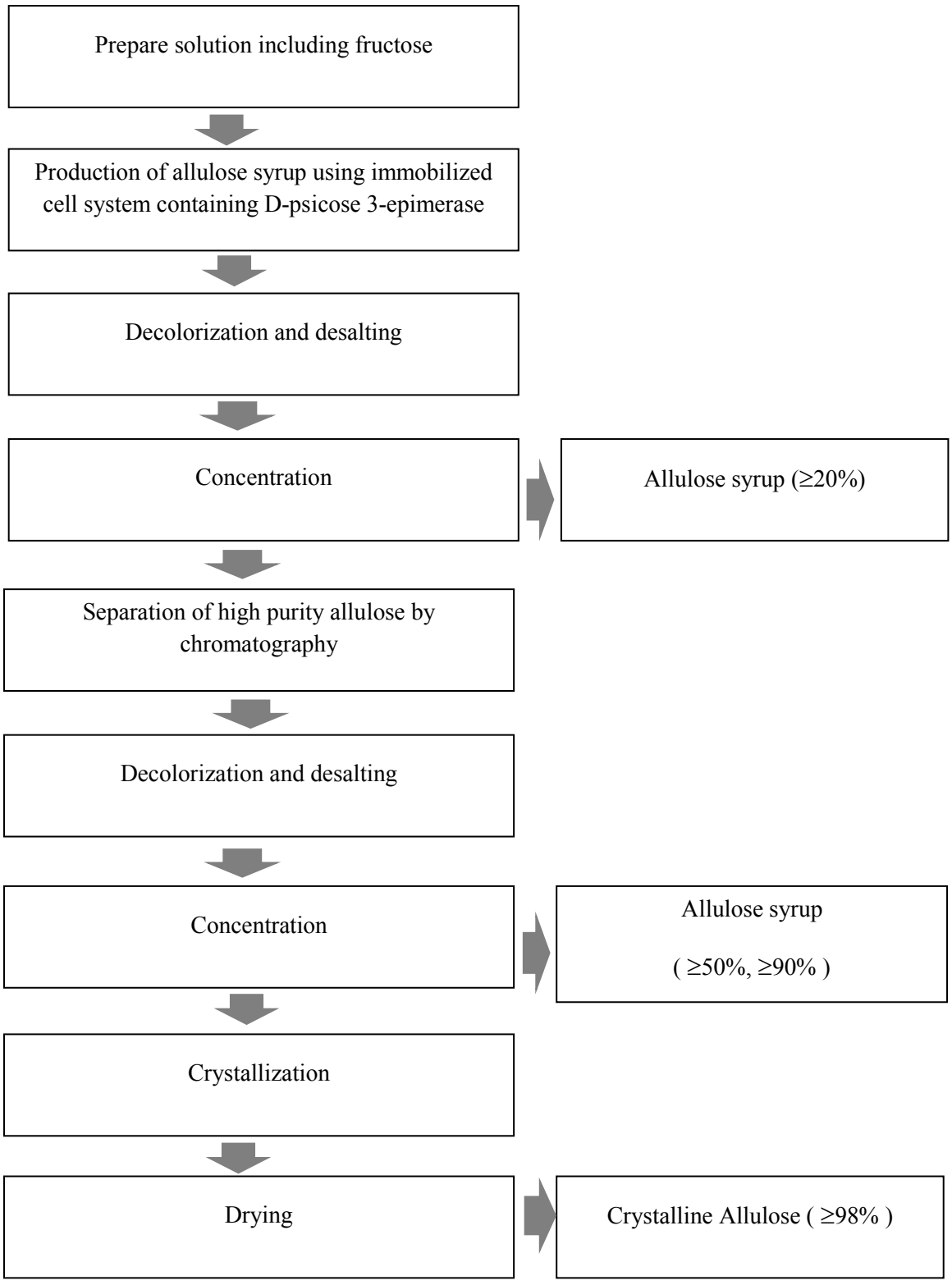


Figure 2. Flow Diagram of Manufacturing Process

2.C. Specifications of D-allulose

As shown in Tables 2-1 to 2-3 and 3-1 to 3-4, the only differences in composition and specification are found in the concentrations of D-allulose, excipients (glucose, fructose and dextrin) and moisture. Specifications for microbial and heavy metal content are the same for powder and liquid forms.

Table 2-1. Composition of Product 1

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	20~25	HPLC
D-fructose*	68~73	HPLC
D-glucose*	4~6	HPLC
Dextrin* (DS2~4)	1~3	HPLC
Protein	ND	AOAC 945.23
Fat	ND	AOAC 996.06
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; D-Allulose + D-Fructose = 93%; D-glucose + Dextrin = 7%; CFU=colony forming units; ND=not detected.

Table 2-2. Composition of Product 2

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	50~55	HPLC
D-fructose*	40~45	HPLC
D-glucose*	1.5~4.0	HPLC
Dextrin* (DS2~4)	1.0~3.5	HPLC
Protein	-	AOAC 945.23
Fat	-	AOAC 996.06
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter

Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; D-Allulose + D-Fructose = 95%; D-glucose + Dextrin = 5%; CFU=colony forming units; ND=not detected.

Table 2-3. Composition of Product 3

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥90	HPLC
Protein	ND	AOAC 945.23
Fat	ND	AOAC 996.06
Moisture	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units; ND=not detected.

Table 3-1. Specifications of Product 1 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥20	HPLC
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01

Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

Table 3-2. Specifications of Product 2 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥50	HPLC
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

Table 3-3. Specifications of Product 3 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥90	HPLC
Moisture	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

Table 3-4. Specifications of Product 4 (Crystalline D-allulose, ≥98%)

Composition	Specification	Analytical Method
Appearance	Powder	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥98	HPLC
Moisture, %, wt/wt	≤2	AOAC 941.14
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.1	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

PART 3. DIETARY EXPOSURE

3.A. Food Sources of D-allulose

As shown in Table 4, D-allulose is a naturally occurring monosaccharide present in small quantities in food products, particularly in selected bakery products, sweets, and fruits (Oshima et al., 2006).

Table 4. D-allulose content in foods (adopted from Oshima et al., 2006)

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
Raisin	38.7
Canned peaches	1.5
Can of mandarin oranges	8.4
Canned cherries	2.0

3.B. Estimated Daily Intakes (EDIs) of Naturally Occurring D-allulose from the Diet

The D-allulose level in each food is not listed in the USDA food composition tables or the National Health and Nutrition Examination Survey (NHANES) databases. Using the dietary content of D-allulose available from the studies of Oshima et al. (2006; Table 3), the EDIs from the diet were estimated. The mean and 90th percentile EDIs of users are 94.8 and 260.7 mg D-allulose/person/day. 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.

Table 5-1. Intake of Naturally Occurring Allulose from the Diet (All Users)

Age, y	N	mg/person/day				mg/kg bw/day				Body wt., kg	
		Mean	SE	P 90	SE	Mean	SE	P 90	SE	Mean	SE
All gender											
1-99 y	8126	94.8	2.5	260.7	12.2	1.46	0.04	3.97	0.12	72.0	0.4
1-6 y	1155	47.0	2.3	117.1	10.7	2.86	0.16	6.93	0.55	17.6	0.2
7-12 y	1074	55.2	3.3	141.0	3.4	1.54	0.09	3.66	0.26	40.5	0.8
13-19 y	1009	99.8	6.7	271.6	10.8	1.53	0.11	4.36	0.30	67.7	1.2
20+ y	4800	104.0	3.0	283.2	11.7	1.28	0.04	3.52	0.16	81.9	0.5
Males											
13-19 y	514	103.8	11.0	284.0	16.6	1.53	0.15	4.44	0.39	72.5	1.2
20+ y	2393	120.7	6.0	295.8	22.5	1.39	0.07	3.89	0.20	88.3	0.6
Females											
13-19 y	495	95.2	14.3	225.2	34.7	1.52	0.22	4.06	0.77	62.5	1.5
20+ y	2407	88.2	3.8	258.9	14.8	1.18	0.04	3.26	0.17	75.8	0.6

BW=body weight; P90=90th percentile; Based on NHANES 2011-2014.

Table 5-2. Intake of Naturally Occurring Allulose from the Diet (Total Population)

Age, y	N	mg/person/day				mg/kg bw/day				Body wt., kg	
		Mean	SE	P 90	SE	Mean	SE	P 90	SE	Mean	SE
All gender											
1-99 y	8126	84.5	2.3	233.8	14.9	1.30	0.04	3.69	0.15	72.0	0.4
1-6 y	1243	44.4	2.2	116.31	10.8	2.71	0.15	6.89	0.56	17.6	0.2
7-12 y	1074	48.8	3.0	136.0	4.8	1.36	0.08	3.45	0.13	40.5	0.8
13-19 y	1009	82.6	9.4	245.5	19.8	1.27	0.12	3.89	0.30	67.7	1.2
20+ y	4800	92.9	2.8	274.2	12.2	1.15	0.03	3.30	0.17	81.9	0.5
Males											
13-19 y	514	89.9	9.8	280.0	13.9	1.33	0.13	4.40	0.48	72.5	1.2
20+ y	2393	107.5	5.1	285.4	17.0	1.24	0.06	3.59	0.24	88.3	0.6
Females											
13-19 y	495	74.9	12.4	198.6	22.4	1.20	0.21	3.39	0.79	62.5	1.5
20+ y	2407	79.1	3.5	216.1	13.6	1.06	0.04	3.00	0.13	75.8	0.6

BW=body weight; P90=90th percentile; Based on NHANES 2011-2014.

3.C. Exposure Estimates Under the Intended Use

3.C.1. EDI of D-allulose Under the Intended Use

The intended use of D-allulose is in the same food products and at levels proportional to those mentioned in the GRN 498 and GRN 400. The results of the EDI assessment are summarized in the two tables below (Tables 6-1 and 6-2). The first table presents the results of the mean of the population as well as the 90th percentile in g/day, and the second in g/kg bw/day (Table 6-1). Since intended use and use levels combined those described in GRN 498 and 400, the EDIs in this GRAS determination are estimated to be slightly higher than those described in the two GRAS notices. However, EDIs presented in this GRAS notice are within the safe intake levels. These results reveal an average maximum exposure would occur in males greater than 19 years of age, with a 90th percentile value of 36.3 g/day or 0.39 g/kg bw/day. On a body weight basis, children aged 2-12 years had shown the highest 90^{th} percentile EDI at 0.50 g/kg bw/day. All subpopulation groups had the EDIs equal to or below 0.5 g/kg bw/day. The toxicity data reveals an LD_{50} of 15.8-16.3 g/kg bw, indicating that even at the highest exposure, D-allulose is not a safety risk.

These estimates are highly amplified since it is not likely that D-allulose will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered. Overall, intended use will result in EDIs at levels significantly below those associated with any potential side effects.

Table 6-1. Maximum EDIs of D-allulose, g/day * (Assuming All the Foods will be Used at the Maximum Use Levels)

		Per User (g/day)		Per Capita (g/day)	
Population	N-user*	Mean	90^{th} Percentile	Mean	90^{th} Percentile
U.S. 2+ y	13,455	11.0	30.0	8.6	24.8
Infants < 2 y	536	0.8	2.6	1.7	4.1
Children 2-12 y	3,223	5.2	14.2	4.1	12.0
Adolescents 13-18 y	1,283	7.6	16.7	5.1	14.6
Males 19+ y	4,178	13.0	36.3	9.8	29.0
Females 19+ y	4,771	12.7	32.6	10.0	29.3

* Based on NHANES 2007-10. U.S.= United States

Table 6-2. Maximum EDIs of D-allulose, g/kg bw/day (Assuming All the Foods will be Used at the Maximum Use Levels) NHANES 2007-10

Population	N-user*	Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
		Mean	90th Percentile	Mean	90th Percentile
US 2+ y	13,455	0.16	0.42	0.12	0.35
Infants < 2 y	536	0.08	0.24	0.15	0.42
Children 2-12 y	3,223	0.19	0.50	0.15	0.42
Adolescents 13-18 y	1,283	0.12	0.29	0.08	0.24
Males 19+ y	4,178	0.14	0.39	0.11	0.31
Females 19+ y	4,771	0.16	0.44	0.13	0.38

* Based on NHANES 2007-2010. BW=body weight.

3.C.2. EDI of Other Components Under the Intended Use

Two D-allulose syrup products (Products 1 and 2) contain other nutrients such as fructose and glucose. Glucose is subjected to 21CFR 184.1277and 168.120. Fructose (in the form of high fructose corn syrup) is subjected to 21CFR 184.1866. Thus, we have not calculated the EDIs of these nutrients from the diet.

PART 4. SELF-LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the D-allulose ingredient.

PART 5. THE 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.

PART 6. BASIS FOR OUR CONCLUSION OF GRAS STATUS

6.A. Current Regulatory Status

The FDA has received two GRAS notices related to food uses of D-allulose (GRN 400 submitted by CJ CheilJedang, Inc., 2011; GRN 498 submitted by Matsutani Chemical, 2014). In these GRAS notices, toxicity-related studies on D-allulose from the literature were presented that support the safety of use of D-allulose. The FDA did not question the acceptability and suitability of these studies to establish the safety of D-allulose for the proposed food uses. The FDA did not have questions on the summary of safety, concluding that D-allulose intake of less than 0.5 g/kg bw/day is safe. Table 4 summarizes previous GRAS notices and the current notice for D-allulose.

Table 7. Summary of Previous and the Current GRAS Notices

GRN	Company	Intended use	EDI, 90th pctl for all users
400	CJ CehilJedang	As a sugar substitute in dietetic or low calorie bakery products, chewing gums, fat-based cream used in modified fat/calorie cookies, cakes and pastries, low calorie hard candies including pressed candy and mints, low calorie frozen dairy desserts, low calorie carbonated beverages, reduced and low calorie non-carbonated beverages, sugar substitutes, low calorie yogurt, medical foods, ready-to-eat cereals (<5% sugar), and coffee mix.	28.5 g/person/day or 0.36 g/kg bw/day
498	Matsutani	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	24.8 g/person/day or 0.33 g/kg bw/day
Present notice	Samyang Corp.	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	30 g/person/day or 0.42 g/kg bw/day

bw= body weight; GRAS= generally recognized as safe; pctl=percentile.

The pertinent information is available as indicated below:
GRN 400: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=400 (page).
GRN 498: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=498 (page).

6.B. Intended Technical Effects

D-allulose will be used as a food ingredient for low calorie and/or dietetic foods due to its technological properties (e.g., functions as a sweetener, humectant, and flavor modifier) and nutritional benefits (such as low calorie and glycemic control).

6.C. Review of Safety Data

As noted above, the FDA has had no question on two GRAS notices related to food uses of D-allulose. The FDA did not have questions on the summary of safety concluding that D-allulose intake up to 0.5 - 0.6 g/kg bw/day is safe. Since the specifications for the liquid and powder forms of D-allulose in this notice are similar to those described in GRN 400 and 498, the metabolism and safety data and other pertinent information discussed in GRN 400 and 498 are applicable to the safety of D-allulose in this GRAS notice. The information is hereby incorporated by reference in these documents and will not be discussed in detail.

Since the FDA's review of GRNs 400 and 498 (GRN 400, FDA, 2012; GRN 498, FDA, 2014), five animal studies were published; one metabolism (Tsukamoto et al., 2014) and four efficacy studies (Hossain et al., 2015; Itoh et al., 2015; Nagata et al., 2015; Ochiai et al., 2014). Findings from these studies were not inconsistent with the agency's prior decision.

6.C.1. Metabolism

A study published since the FDA's decision of 2014 confirmed the previous findings that D-allulose was rapidly excreted through urine (Tsukamoto et al., 2014). Following oral administration, D-allulose is partly absorbed in the digestive tract and enters the bloodstream. The maximum blood concentration (48.5±15.6 μg/g) was observed at 1 hour. Excretion via urine was 20% within 1 hour and 33% within 2 hours (Tsukamoto et al., 2014). Accumulation in organs was detected only in the liver. Following intravenous administration, blood concentration of D-allulose was decreased with the half-life of 57 minutes, and the excretion via urine reached almost 50% within 1 hour. Seven days after the single-dose oral administration, the remaining amounts in the whole body was less than 1%.

Previously reviewed studies reported that about 98% of intravenously administered D-allulose is excreted in the urine within 6 h (Whistler et al., 1974). When orally ingested, urinary excretion of unchanged D-allulose ranged from 11 to 25% (Matsuo et al., 2003). The data indicate that D-allulose 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-allulose is fermented to short chain fatty acids (SCFA) by intestinal microflora in the colon (Noda and Oh, 1992) or is excreted in the feces (Matsuo et al., 2004).

6.C.2. Animal Toxicity Studies

Since the FDA's last review of D-allulose in 2012-2014 (GRNs 400 and 498; U.S. FDA, 2012 and 2014, respectively), one new paper has been published (Nishii et al., 2016a). This study reported that a single oral dose of 1 or 4 g/kg bw did not cause any treatment-related abnormalities in dogs. All dogs were active and had a good appetite throughout the study period. Blood glucose concentration slightly decreased without a rise in plasma insulin concentration 2 h after D-allulose administration. Plasma alkaline phosphatase activities showed a mild increase

between 12 and 48 h after D-allulose administration. These data suggested that a single oral dose of D-allulose does not show severe toxicity in dogs.

Previous reviews included the LD_{50} value of D-allulose in rats at 15.8-16.3 g/kg bw (Matsuo et al., 2002). Subacute toxicity studies (up to 34 days) in rats showed that D-allulose concentration of up to 20% of the diet did not show adverse effects (Table 4; Matsuo et al., 2002).

A 90 day subchronic toxicity study reported the no observed adverse effect level (NOAEL) for D-allulose as 3% of diet, the highest level tested (Matsuo et al., 2012). A 12-18 month chronic toxicity study showed that D-allulose at the dose of 3% D-allulose in the diet (or 1,280 mg/kg bw/day), the highest level tested, did not show adverse effects (Yagi and Matsuo, 2009).

In summary, D-allulose, like other monosaccharides, belongs to the group that has the lowest toxicity rating and is classified as an ordinary carbohydrate substance. Thus, the use of D-allulose in foods and beverages is not expected to pose a safety concern.

Table 8. Summary of Animal Toxicity Studies Referenced in GRNs 400 and 498

Species	Dosage	Duration	Primary endpoints and NOAEL	Reference
Dogs	1 and 4 g/kg bw	Single dose	Acute toxicity-food intake and selected clinical chemistry	Nishii et al., 2016a
Male rats	8, 11, 14, 17, and 20 g/kg bw (D-allulose in water)	Single dose	Acute toxicity-LD_{50}, 16.3 g/kg bw	Matsuo et al., 2002
Young rats	10, 20, 30, and 40% in the diet	34 days	Feed intake, wt. gain, and organ wt.; NOAEL-up to 20% in the diet (corresponding to 10,000 mg/kg bw/day)	Matsuo et al., 2002
Male Wistar rats	3% in the diet	90 days	Feed intake, wt gain, organ wt., serum biochemistry, hematology, and histology; NOAEL- 3% in diet, the highest level tested	Matsuo et al., 2012
36 Male rats, Wistar	3% in the diet or 1,280 mg/kg bw/d (control, 3% sucrose)	12-18 months	Feed and energy intakes, wt. gain, organ wt., digestive tract size, serum biochemistry, hematology, and histology; NOAEL- 1,280 mg/kg bw/day, the highest level tested	Yagi and Matsuo, 2009

bw= body weight; NOAEL= no observed adverse effect level; wt= weight.

6.C.3. Animal Efficacy Studies Reporting No Adverse Effects of D-allulose

Since the FDA's last review of D-allulose (GRNs 400 and 498; U.S. FDA, 2012 and 2014, respectively), six animal efficacy studies were published based on the repeat dose

administration of D-allulose at high dietary concentrations for long durations (Table 6; Han et al., 2016; Hossain et al., 2015; Itoh et al., 2015; Nagata et al., 2015; Nishii et al., 2016b; Ochiai et al., 2014). No studies reported results inconsistent with the FDA's prior reviews of 2012-2014. Although these studies were designed to investigate the efficacy of D-allulose on various health parameters, several safety related endpoints were obtained during the experiments. Therefore, these studies are reviewed below as additional supporting information.

Recent efficacy studies showed that D-allulose at the level of up to 5% in the diet (corresponding to up to 2,500 mg/kg bw/day) did not cause any adverse effects on food efficiency, glucose metabolism, lipid metabolism, inflammatory biomarkers, body fat accumulation, and/or histopatholgical parameters (Han et al., 2016; Hossain et al., 2015; Itoh et al., 2015; Nagata et al., 2015; Nishi et al., 2016 b; Ochiai et al., 2014).

Nishii et al. (2016b) reported that oral administration of D-allulose (0.2 g/kg bw) decreased plasma glucose concentrations after oral glucose or maltose administration, with a diminished plasma insulin rise in dogs. However, D-allulose showed no effect on plasma glucose and insulin concentrations after feeding. The data suggest that D-allulose administration may be beneficial in dogs with impaired glucose tolerance.

In a study by Han et al. (2016), mice were fed a high fat diet with or without various sugar substitutes (d-glucose, d-fructose, erytritol, or D-allulose, n = 10 per group) for 16 wk. Body weight and fat-pad mass in the D-allulose group were dramatically lowered to that of the normal group with a simultaneous decrease in plasma leptin and resistin concentrations. D-allulose lowered plasma and hepatic lipids while elevating fecal lipids. In the liver, activities of both fatty acid synthase and β-oxidation were downregulated by D-allulose to that of the normal group; however, in white adipose tissue (WAT), fatty acid synthase was decreased while β-oxidation activity was enhanced. No adverse effects of D-allulose were reported.

Long-term administration (60 weeks) of D-allulose at a dose of 5% of the diet prevented the commencement and progression of type 2 diabetes through the maintenance of blood glucose levels and the control of postprandial hyperglycemia with decreased levels of HbA_{1c} (by ~50%) in comparison to control rats (Hossain et al., 2015). This improvement in glycemic control was accompanied by the maintenance of plasma insulin levels and the preservation of pancreatic β-cells with a significant reduction in inflammatory markers. In the control group, the glucose levels started to increase slowly from 25 weeks and then sharply until 60 weeks, whereas in the allulose group the glucose levels started to increase slightly from 45 weeks and remained constant until 60 weeks. By the end of 60 weeks, the fasting blood glucose concentrations in the psicose group were approximately 35% lower than that of the control group. Body fat accumulation, in particular adipose tissue, was lower (by ~25-30%) in the treatment group, with decreased infiltration of macrophages in the abdominal adipose tissue. No adverse effects of D-allulose were reported.

The study by Itoh et al. (2015) also reported anti-obesity effects of D-allulose (0, 2.5, or 5% of the diet or 1,500-2,000 or 3,000-4,000 mg/kg bw/day) in inherited leptin-deficient ob/ob mice. Wild type C57BL/6J mice were used as an animal control (0% D-allulose). The results of this study showed that subchronic ingestion for 15 weeks significantly decreased body weights (by ~20%), liver weights (by ~6%), and total fat mass (by ~7%), including abdominal visceral fat

(by ~5%) in the 5% allulose group. During the 15-week period, the total calorie intake of the 5% D-allulose treatment significantly decreased by 10% compared to that observed in both the control and 2.5% D-allulose groups. Furthermore, D-allulose improved hepatic steatosis as evaluated using hepatic histological evaluation and magnetic resonance imaging (MRI). In control mice, fat deposition produced a severely damaged liver histology presenting as remarkable ballooning degeneration. The ballooning degeneration and hepatic steatosis improved after the subchronic ingestion of D-allulose. The authors concluded that D-allulose may be useful as a supplement for preventing and improving obesity and obesity-related disorders. No adverse effects of D-allulose were reported.

In a study by Nagata et al. (2015), effects of D-allulose on lipid metabolism were evaluated. Rats were fed diets with or without 3% D-allulose for 4 weeks. In experiment 1, feeding D-allulose significantly decreased body weight by approximately 5%, but not food intake. Liver enzyme activities involved in lipogenesis were significantly lowered by the D-allulose diet, whereas gene expression of a transcriptional modulator of fatty acid oxidation was enhanced. Rats fed D-allulose had significantly lower serum insulin and leptin levels. In experiment 2, feeding the D-allulose diet resulted in significantly lower body weight (389±3 vs. 426±6 g, $p < 0.05$) and food intake (23.8±0.2 vs. 25.7±0.4 g/day, $p < 0.05$) compared to the control diet. Rats fed the D-allulose diet had significantly higher energy expenditure in the light period and fat oxidation in the dark period compared to rats fed the control diet, whereas carbohydrate oxidation was lower. The results indicate that the D-allulose diet decreased lipogenesis, increased fatty acid oxidation, and enhanced 24 h energy expenditure, leading to D-allulose's potential for weight management. No adverse effects of D-allulose were reported.

These studies confirmed the previous findings that D-allulose at the level of up to 5% in the diet did not cause treatment-related abnormalities on measured outcomes (Table 6; Baek et al., 2010; Chung et al., 2012a; Hossain et al., 2012; Matsuo et al., 2001a, 2001b; Matsuo and Izumori, 2004, 2006, 2009; Ochiai et al., 2013).

Several mechanisms of actions have been proposed to explain potential mechanisms of anti-obese and anti-hyperglycemic effects of D-allulose (Previous GRNs covered most of these aspects):

1) its zero-calorie effects and 70% relative sweetness of sucrose,
2) the inhibition of enzymatic activities for the digestion of polysaccharides, such as glucoamylase and maltase (Iida et al., 2008; Matsuo and Izumori, 2006),
3) inhibition of hepatic fatty acid synthatase (Matsuo et al., 2001a, 2001b),
4) the preservation of pancreas β-cells through the suppression of proinflammatory cytokines and reactive oxygen species production (Hossain et al., 2015),
5) decreased absorption of sugars (Baek et al., 2010; Matsuo and Izumori, 2009),
6) enhanced insulin sensitivity (Hossain et al., 2012; Iida et al., 2008) and/or
7) altered hepatic glucose metabolism via the translocation of glucokinase (Hossain et al., 2011).

Animal efficacy studies are summarized in Table 9. None of the animal efficacy studies reported adverse effects of D-allulose. For these 'pivotal' studies, the dose levels represent the maximum doses administered, rather than absolute safety endpoints.

Table 9. Animal Efficacy Studies Reporting No Adverse Effects of D-allulose

Species	Dosage	Length	Primary endpoints	Reference
Recent Animal Efficacy Studies				
Dogs	0.2 g/kg bw	Single dose	Blood glucose and insulin parameters	Nishii et al., 2016b
Mice	5% of high fat diet	16 weeks	Body weight, plasma concentrations of leptin and resistin, plasma and hepatic levels of lipids, and fecal excretion of lipids	Han et al., 2016
Young male Wistar rats	5% of high sucrose diet or control diet	8 weeks	Feed intake, weight gain, clinical chemistry, energy expenditure, and body fat accumulation	Ochiai et al., 2014
Diabetic rats	5% of diet	60 weeks	Body weight gain, glucose metabolism, inflammatory biomarkers, and abdominal fat deposition.	Hossain et al., 2015
Rat, Sprague Dawley	3% of diet	4 weeks	Lipid metabolism (serum and liver lipid levels, liver enzyme activity, and gene expression), body weight	Nagata et al., 2015
Mice (ob/ob and wild type C57BL/6J)	0, 2.5, or 5% of diet	15 weeks	Body and fat weights, liver weights, and hepatic steatosis	Itoh et al., 2015
Studies Referenced in GRNs 400 and 498				
Rat, Sprague-Dawley	5% of high fat diet	8 weeks	Feed intake, weight gain, liver weight, visceral fat mass, blood lipid profile	Chung et al., 2012a
Male Wistar rats	5% of high sucrose diet or high starch diet	8 weeks	Body weight, food intakes, organ weight, serum clinical chemistry, liver triglycerides, carbohydrates and glycogen, and body fat	Ochiai et al., 2013
Diabetic rats	5% of diet	13 weeks	Body weight, glucose metabolism, inflammatory biomarkers, and abdominal fat deposition.	Hossain et al., 2012
Male mice	0.2 g/kg bw/d	4 weeks	Glycemic responses, insulin release, and blood lipid profiles, 0.2 g/kg bw/day	Baek et al., 2010
24 Male rats, Wistar	5% in the high (25%) and low fat (5%) diets	16 weeks	Body weight, energy intake, body fat, organ wt., glucose tolerance, serum adipocytokine concentrations (adiponectin, tumor necrosis factor alpha, leptin), and liver glycogen and triglycerides.	Matsuo and Izumori, 2004
Male rat	5% in the diet	3 weeks	Body fat and lipid metabolism	Matsuo et al., 2001a

Male rat	5% in the diet	4 weeks	Body fat and lipid metabolism	Matsuo et al., 2001b
Male rat	5% in the diet	8 weeks	Body fat and glycemic responses	Matsuo and Izumori, 2006
Male rat	2,000 mg/kg bw	Single dose	Body fat and glycemic responses	Matsuo and Izumori, 2009

bw= body weight; d= day

6.C.4. Human Clinical Studies

Since the FDA's last review of D-allulose in 2014 (GRNs 400 and 498; U.S. FDA, 2012 and 2014, respectively), no new literature has been published. Several human clinical studies previously reviewed reported no adverse effects of D-allulose (Table 6; Hayashi et al., 2010; Iida et al., 2007, 2008, 2010). Like non-digestible oligosaccharides and fiber ingredients, the only known side effect of D-allulose is gastrointestinal discomfort when ingested in large quantities. Even if gastrointestinal discomfort is noted when consumed in large quantities of D-allulose, it is not considered to be of toxicological significance since this type of symptom is usually transient and is often associated with ingestion of non-digestible carbohydrates including dietary fiber (IOM, 2002).

A clinical study showed that the maximum tolerable levels in humans were 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females, with the mean value of 0.55 g/kg bw/day (Table 10). These dosages correspond to 33.3 g/day for a 67 kg Asian male and 31.0 g/day for a 52 kg Asian female (Iida et al., 2007). These dosages also correspond to 45 - 46 g/person/day for an average American adult aged 20 years or older.

Table 10. Human Clinical Studies Referenced in GRNs 400 and 498

Dosage	Length	Results	Reference
Up to 0.9 g/kg bw/d	6 days	No gastrointestinal symptoms up to 0.5 - 0.6 g/kg bw/d	Iida et al., 2007
15 g/d (5 g in tea, three times a day)	12 weeks	Positive impact on glycemic responses; no adverse effects were noted.	Hayashi et al., 2010
7.5 g in beverage	Single dose	Positive impact on glycemic and insulinemic responses; no adverse effects were noted.	Iida et al., 2008
Up to 340 mg/kg bw in beverage	Single dose	Metabolism study; no adverse effects were noted.	Iida et al., 2010

bw= body weight; d=day

6.D. SUMMARY

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

D-allulose has been safely used as a food ingredient around the world for a decade. As a result, a number of comprehensive reviews of the safety of D-allulose have been published (Chung et al., 2012b). In addition, the FDA has had no question on two GRAS notices related to the safety of D-allulose (GRN 400, FDA 2012; GRN 498, FDA, 2014).

6.D.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. Samyang Corp.'s D-allulose is manufactured under cGMP using common food industry materials and processes. Samyang Corp. uses a 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 concerning the chemistry of D-allulose. This GRAS determination is based on the data and information generally available and consented opinion about the safety of D-allulose. The literature indicates that D-allulose offers consumers benefits without adverse effects.

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 and meets all applicable purity standards.
2. Samyang Corp.'s D-allulose will be used as a sugar substitute and/or as a flavor modifier in food applications at use levels ranging from 2 to 100% in: selected bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics), beverages (non-alcoholic, low or reduced calorie, sugar free); cereals; chewing gums; confections and frostings; frozen dairy desserts (ice cream, soft serve, sorbet; low calorie, reduced calorie, sugar-free); yogurt and frozen yogurt (low calorie, reduced calorie, sugar-free); dressings for salads; gelatins, pudding and fillings (low calorie, reduced calorie, sugar-free); hard and soft candies (low calorie, reduced calorie, sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low calorie, reduced calorie, sugar-free) and fat based cream.
3. The LD_{50} value of D-allulose in rats is 15.8-16.3 g/kg. A chronic toxicity study 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 3% of the diet, the highest level tested.
4. A human clinical study showed that the maximum tolerable levels in humans were 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females. The only side effect of non-digestible carbohydrates, including D-allulose, is gastrointestinal discomfort when ingested in large quantities. This type of symptom is usually transient and is not considered to be of toxicological significance (IOM, 2002).
5. The proposed food use results in exposure at levels below those associated with any adverse effects. The EDI assessments are based on the assumption that Samyang Corp.'s D-allulose will replace currently marketed D-allulose. Thus, cumulative exposures are not expected. In addition, the EDIs presented in this notice are highly amplified estimates.

6. In the previous GRAS notices (GRN 400 and 498) to the FDA, the safety of D-allulose has been established in animal toxicity studies and mutagenicity studies, and is further supported by human clinical studies.
7. Additional animal studies published subsequent to the FDA GRAS notices continue to support the safety of D-allulose as a food ingredient.

Overall, there are no indications of significant adverse effects related to D-allulose in the publicly available literature. 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.E. 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-allulose preparations in foods and beverages, meeting appropriate specifications and used according to cGMP, is GRAS.

PART 7. DATA AND INFORMATION ARE GENERALLY AVAILABLE

7.1. DATA AND INFORMATION ARE GENERALLY AVAILABLE

All the references including animal and human studies are generally available.

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

Chung YM, Hyun Lee J, Youl Kim D, Hwang SH, Hong YH, Kim SB, Jin Lee S, Hye Park C. Dietary D-allulose reduced visceral fat mass in high-fat diet-induced obese rats. J Food Sci. 2012a;77:H53-H58.

Chung MY, Oh DK, Lee KW. Hypoglycemic health benefits of D-psicose. J Agric Food Chem. 2012b;60:863-9.

FDA. 2012. GRN 400. D-psicose, filed by CJ CheilJedang.

FDA. 2014. GRN 498, D-allulose (synonymous with D-psicose), filed by Matsutani Chemical Industry.

Han Y, Han HJ, Kim AH, Choi JY, Cho SJ, Park YB, Jung UJ, Choi MS. d-Allulose supplementation normalized the body weight and fat-pad mass in diet-induced obese mice via the regulation of lipid metabolism under isocaloric fed condition. Mol Nutr Food Res. 2016;60:1695-1706.

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-allulose in borderline diabetes and the safety of long-term ingestion by normal human subjects. Biosci Biotechnol Biochem. 2010;74:510-519.

Hossain MA, Kitagaki S, Nakano D, Nishiyama A, Funamoto Y, Matsunaga T, Tsukamoto I, Yamaguchi F, Kamitori K, Dong Y, Hirata Y, Murao K, Toyoda Y, Tokuda M. Rare sugar D-psicose improves insulin sensitivity and glucose tolerance in type 2 diabetes Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Biochem Biophys Res Commun. 2011;405:7-12.

Hossain A, Yamaguchi F, Matsunaga T, Hirata Y, Kamitori K, Dong Y, Sui L, Tsukamoto I, Ueno M, Tokuda M. Rare sugar D-allulose protects pancreas β-islets and thus improves insulin resistance in OLETF rats. Biochem Biophys Res Commun. 2012;425:717-723.

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-allulose prevents progression and development of diabetes in T2DM model Otsuka Long-Evans Tokushima Fatty rats. Drug Design Devel Ther. 2015;9:525-535.

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 Advanced Food Ingred. 2007;10: 15-19.

Iida T, Kishimoto Y, Yoshikawa Y, Hayashi N, Okuma K, Tohi M, Yagi K, Matsuo T, Izumori K. Acute D-allulose administration decreases the glycemic responses to an oral maltodextrin tolerance test in normal adults. J Nutr Sci Vitaminol. 2008;54:511-514.

Iida T, Hayashi N, Yamada T, Yoshikawa Y, Miyazato S, Kishimoto Y, Okuma K, Tokuda M, Izumori K. Failure of D-allulose absorbed in the small intestine to metabolize into energy and its low large intestinal fermentability in humans. Metabolism. 2010;59:206-214.

Institute of Medicine (IOM). 2002. Dietary Reference Intakes for energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein, and amino acids. National Academy Press, Washington, DC.

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:H1619-1626.

Karabinos JV. D-allulose, sorbose and tagatose. Adv Carbohydr Chem. 1952;7:99-136.

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;10:233-237.

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;48:512-516.

Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Metabolic effects of D-allulose in rats: Studies on fecal and urinary excretion and cecal fermentation. Asia Pac J Clin Nutr. 2003;12:225-231.

Matsuo T. 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;53:453-460.

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;53:453-460.

Matsuo T, Izumori K. D-allulose inhibits intestinal α-glucosidase and suppresses glycemic response after carbohydrate ingestion in rats. Tech Bull Fac Agr., Kagawa Univ. 2006;58:27–32.

Matsuo T, Izumori K. D-Psicose inhibits intestinal alpha-glucosidase and suppresses the glycemic response after ingestion of carbohydrates in rats. J Clin Biochem Nutr. 2009;45:202-206.

Matsuo T, Ishii R, Shirai Y. The 90-day oral toxicity of D-allulose in male Wistar rats. J Clin Biochem Nutr. 2012;50:158-161.

Nagata Y, Kanasaki A, Tamaru S, Tanaka K. d-D-allulose, an epimer of d-fructose, favorably alters lipid metabolism in Sprague-Dawley rats. J Agric Food Chem. 2015;63:3168-3176.

Nishii N, Nomizo T, Takashima S, Matsubara T, Tokuda M, Kitagawa H. Single oral dose safety of D-allulose in dogs. J Vet Med Sci. 2016;78:1079-1083.

Nishii N, Nomizo T, Takashima S, Matsubara T, Tokuda M, Kitagawa H. Effects of D-allulose on glucose metabolism after the administration of sugar or food in healthy dogs. J Vet Med Sci. 2016;78:1657-1662.

Noda K, Oh T. Metabolism and disposition of erythritol after oral administration in rats. J Nutr. 1992; 122:1266-1272.

Ochiai M, Nakanishi Y, Yamada T, Iida T, Matsuo T. Inhibition by dietary D-allulose of body fat accumulation in adult rats fed a high-sucrose diet. Biosci Biotechnol Biochem. 2013;77:1123-1126.

Ochiai M, Onishi K, Yamada T, Iida T, Matsuo T. D-allulose increases energy expenditure and decreases body fat accumulation in rats fed a high-sucrose diet. Int J Food Sci Nutr. 2014; 65:245-250.

Oshima H, Kimura I, Izumori K. D-allulose contents in various food products and its origin. Food Sci Technol Res. 2006;12:137-143.

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-allulose. Drug Design Devel Ther. 2014;8:1955-1964.

Whistler RL, Singh PP, Lake WC. D-allulose metabolism in the rat. Carbohydr Res. 1974; 34: 200-202.

Yagi K, Matsuo T. The study on long-term toxicity of D-allulose in rats. J Clin Biochem Nutr. 2009;45:271-277.

7.2. DATA AND INFORMATION ARE NOT GENERALLY AVAILABLE

Not applicable.

APPENDIX A. CERTIFICATE OF ANALYSIS

1. Product 1. D-allulose syrup

Composition	Lot 1 (2015.08.08)	Lot 2 (2015.08.09)	Lot 3 (2015.08.20)	Analytical Method
Brix	75 Brix (%)			Brixmeter
pH	3.0 ~ 7.0			pH meter
D-Allulose*	24.63%	25.16%	25.04%	HPLC
Moisture	< 25%			AOAC941.14
Fructose or other sugars*	75.37%	74.84%	74.96%	HPLC
Total plate count	Negative	Negative	Negative	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0095 ppm	0.0048 ppm	0.0063 ppm	AOAC 2015.01
As	0.0071 ppm	0.0014 ppm	0.0024 ppm	AOAC 2015.01
Cd	0.0020 ppm	0.0011 ppm	0.0027 ppm	AOAC 2015.01

*Dry weight basis.

2. Product 2. D-allulose syrup

Composition	Lot 1 (2015.09.15)	Lot 2 (2015.09.30)	Lot 3 (2015.10.20)	Analytical Method
Brix	75 Brix (%)			Brixmeter
pH	3.0 ~ 7.0			pH meter
D-Allulose*	53.37%	53.22%	54.95%	HPLC
Moisture	< 25%			AOAC941.14
Fructose or other sugars*	46.63%	46.78%	45.05%	HPLC
Total plate count	Negative	Negative	Negative	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0040 ppm	0.0033 ppm	0.0074 ppm	AOAC 2015.01

As	0.0015 ppm	0.0015 ppm	0.0024 ppm	AOAC 2015.01
Cd	0.0038 ppm	0.0016 ppm	0.0013 ppm	AOAC 2015.01

*Dry weight basis.

3. Product 3. D-allulose syrup

Composition	Lot 1 (2015.09.15)	Lot 2 (2015.8.28)	Lot 3 (2015.10.06)	Analytical Method
Brix	75 Brix (%)			Brixmeter
pH	3.0 ~ 7.0			pH meter
D-Allulose*	95.90%	95.25%	96.19%	HPLC
Moisture	< 25%			AOAC 941.14
Fructose or other sugars*	4.10%	4.75%	3.81%	HPLC
Total plate count	Negative	Negative	Negative	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0024 ppm	0.0021 ppm	0.0028 ppm	AOAC 2015.01
As	0.0011 ppm	0.0006 ppm	0.0018 ppm	AOAC 2015.01
Cd	0.0022 ppm	0.0012 ppm	0.0014 ppm	AOAC 2015.01

*Dry weight basis.

4. Product 4-Crystalline D-**a**llulose, ≥98%

Composition	Lot 1 (2015.09.15)	Lot 2 (2015.9.30)	Lot 3 (2015.10.20)	Analysis Method
Moisture	0.15%	0.16%	0.14%	AOAC 941.14
D-Allulose*	99.44%	99.03%	99.43%	HPLC
Fructose or other sugars*	0.41%	0.81%	0.43%	HPLC
Total plate count	2.0×10^2	2.7×10^2	2.0×10^2	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0065 ppm	0.0054 ppm	0.0017 ppm	AOAC 2015.01

As	0.0027 ppm	0.0059 ppm	0.0062 ppm	AOAC 2015.01
Cd	0.0014 ppm	0.0016 ppm	0.0011 ppm	AOAC 2015.01

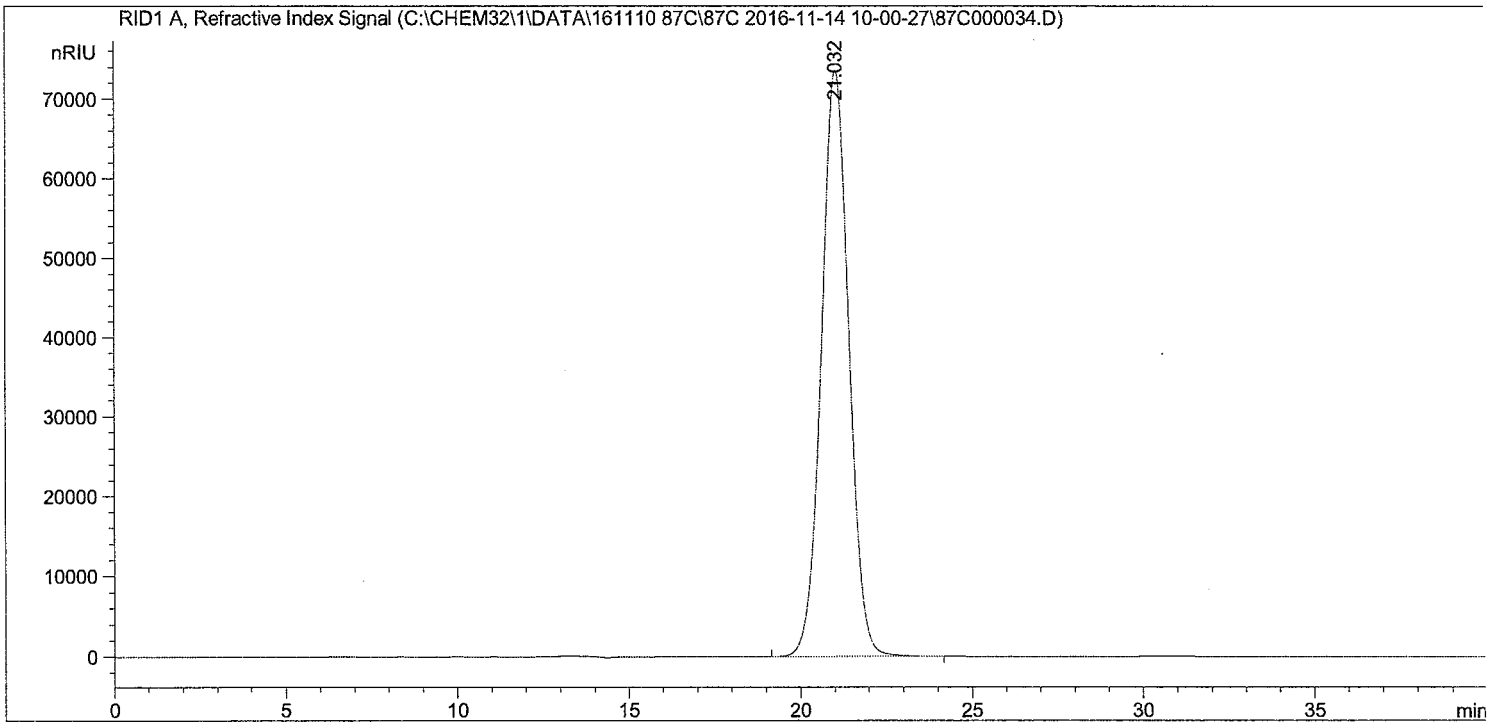
*Dry weight basis.

Data File C:\CHEM32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C000034.D
Sample Name: psicose

pure allulose

==========

Acq. Operator : jinsol Seq. Line : 34
Acq. Instrument : Instrument 1 Location : Vial 10
Injection Date : 11/15/2016 12:41:44 AM Inj : 1
Inj Volume : 10 µl
Acq. Method : C:\Chem32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C-40MIN.M
Last changed : 1/21/2016 10:53:59 AM by Kwon sg
Analysis Method : C:\CHEM32\1\METHODS\87C-30MIN.M
Last changed : 11/15/2016 8:27:12 AM by Goeun
(modified after loading)



==========

Area Percent Report

==========

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

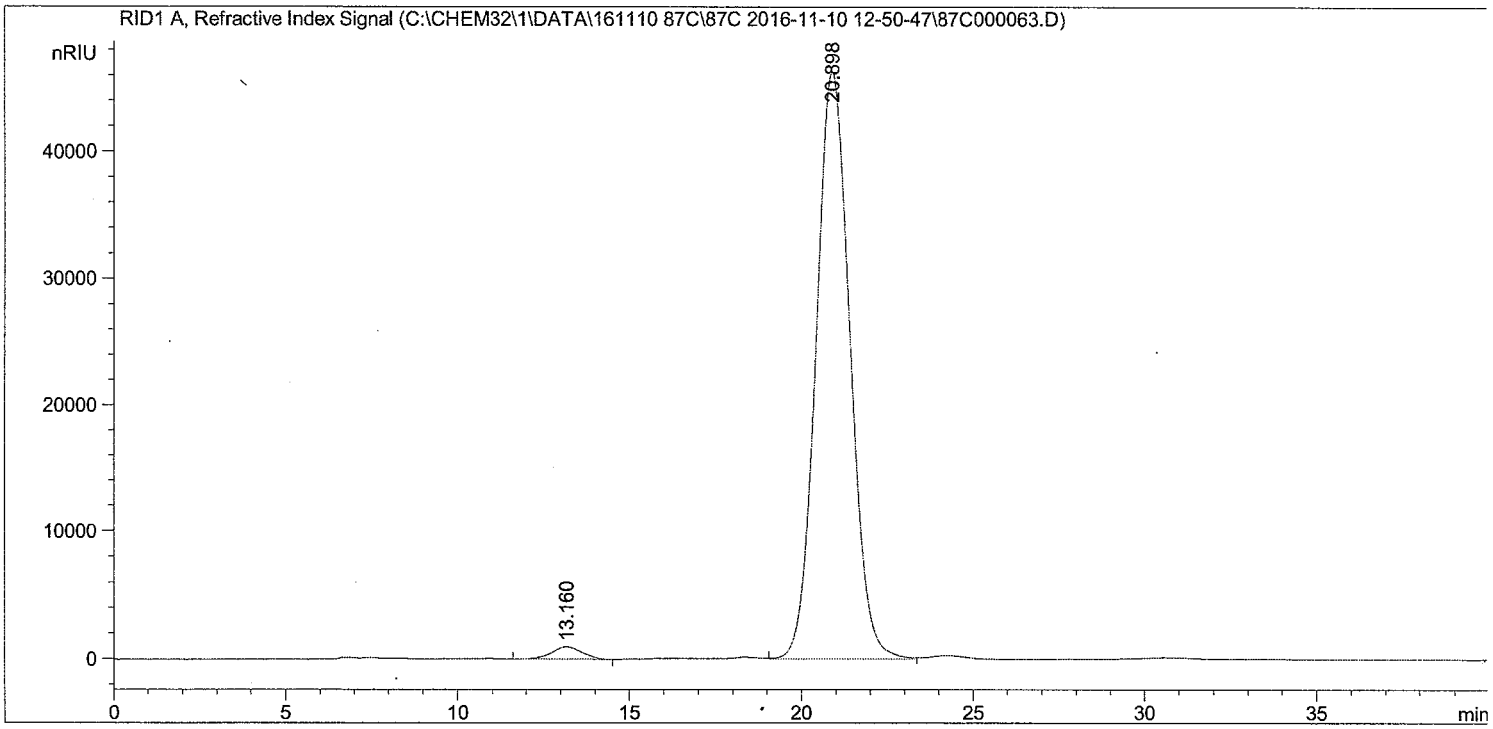
Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	21.032	BB	0.8641	4.09187e6	7.36550e4	100.0000
Totals :				4.09187e6	7.36550e4	

Data File C:\CHEM32\1\DATA\161110 87C\87C 2016-11-10 12-50-47\87C000063.D
Sample Name: allulose 90

90% allulose

Acq. Operator	: jinsol	Seq. Line	: 63
Acq. Instrument	: Instrument 1	Location	: Vial 91
Injection Date	: 11/11/2016 3:58:35 PM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: C:\Chem32\1\DATA\161110 87C\87C 2016-11-10 12-50-47\87C-40MIN.M		
Last changed	: 1/21/2016 10:53:59 AM by Kwon sg		
Analysis Method	: C:\CHEM32\1\METHODS\87C-30MIN.M		
Last changed	: 11/15/2016 8:27:12 AM by Goeun		
	(modified after loading)		



Area Percent Report

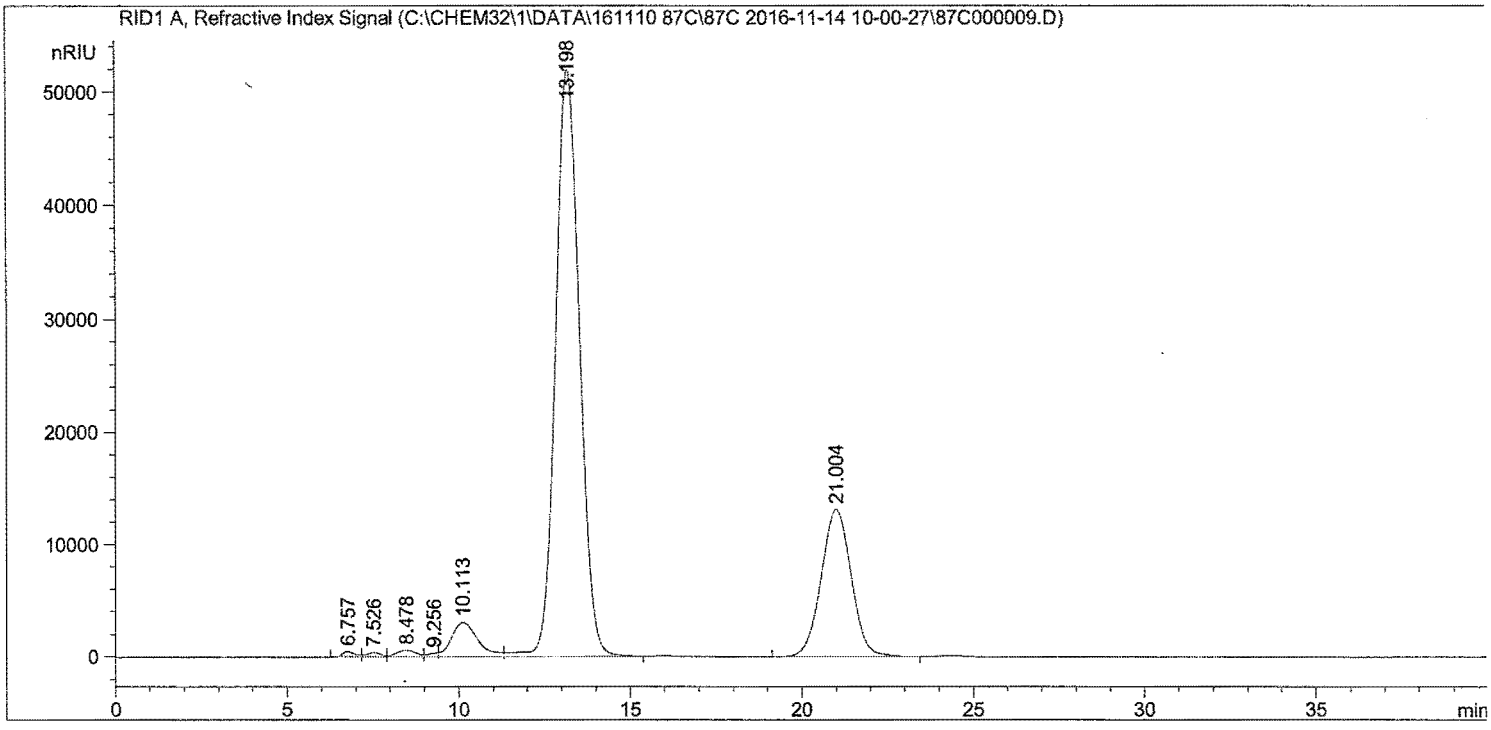
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	13.160	VV	0.9658	6.33221e4	985.33160	1.9581
2	20.898	VV	1.0665	3.17061e6	4.63141e4	98.0419
Totals :				3.23393e6	4.72994e4	

Data File C:\CHEM32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C000009.D
Sample Name: alluloase 20-P

```
Acq. Operator    : jinsol                          Seq. Line :   9
Acq. Instrument  : Instrument 1                     Location : Vial 94
Injection Date   : 11/14/2016 1:40:23 PM                Inj :   1
                                                  Inj Volume : 10 µl
Acq. Method      : C:\Chem32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C-40MIN.M
Last changed     : 1/21/2016 10:53:59 AM by Kwon sg
Analysis Method  : C:\CHEM32\1\METHODS\87C-30MIN.M
Last changed     : 11/15/2016 8:27:12 AM by Goeun
                   (modified after loading)
```



Area Percent Report

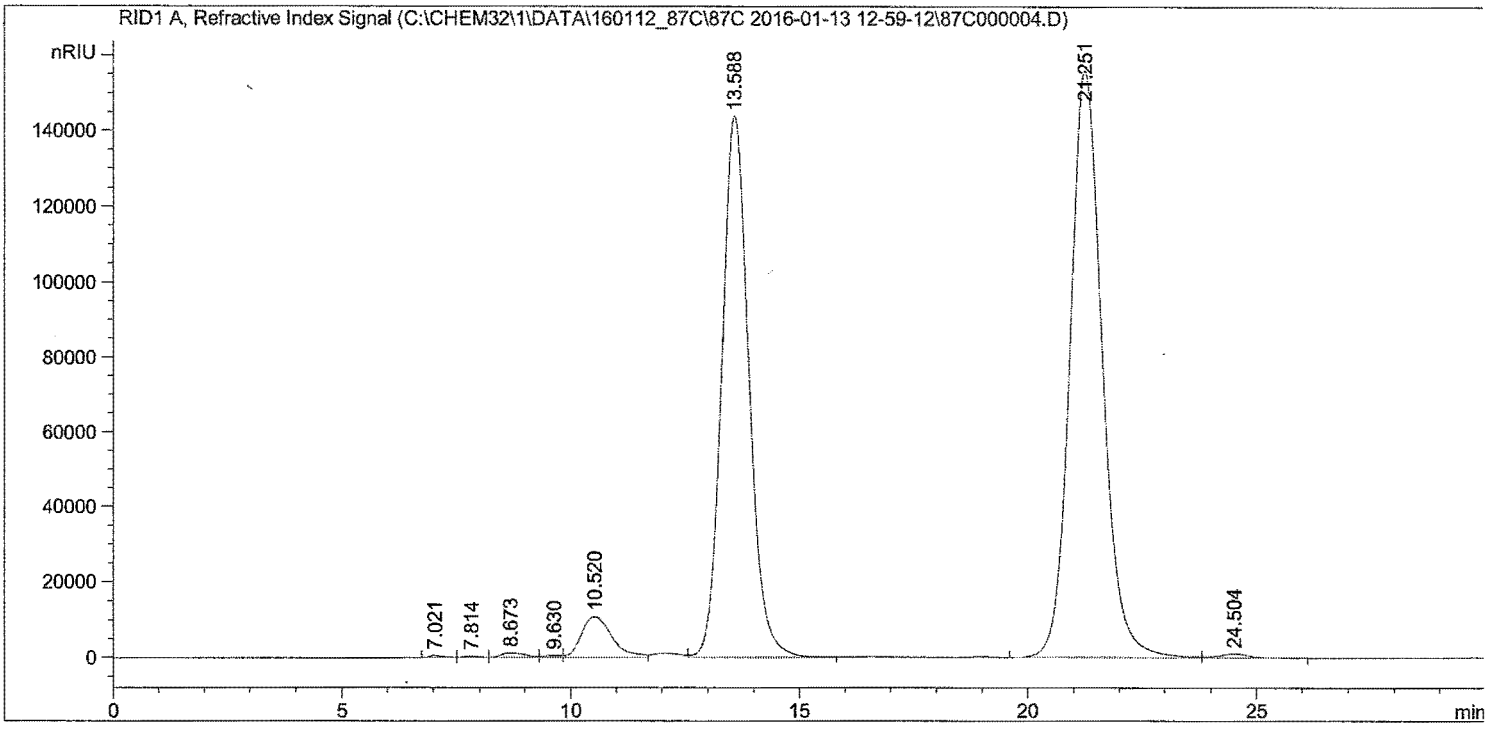
```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	6.757	BV	0.3905	1.29029e4	495.07358	0.3629
2	7.526	VV	0.4332	1.17543e4	405.56537	0.3306
3	8.478	VV	0.6412	2.47657e4	605.62048	0.6965
4	9.256	VV F	0.3317	8050.79541	335.67728	0.2264
5	10.113	VV	0.8285	1.63481e5	3028.44727	4.5980

Data File C:\CHEM32\1\DATA\160112_87C\87C 2016-01-13 12-59-12\87C000004.D
Sample Name: 50%-1

```
Acq. Operator    : Kwon sg                          Seq. Line :   4
Acq. Instrument  : Instrument 1                      Location : Vial 4
Injection Date   : 1/13/2016 2:41:56 PM                  Inj :   1
                                                   Inj Volume : 10 µl
Acq. Method      : C:\Chem32\1\DATA\160112_87C\87C 2016-01-13 12-59-12\87C-30MIN.M
Last changed     : 1/13/2016 12:59:10 PM by Kwon sg
Analysis Method  : C:\CHEM32\1\METHODS\87C-30MIN.M
Last changed     : 11/15/2016 3:58:36 PM by Goeun
                   (modified after loading)
```



Area Percent Report

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

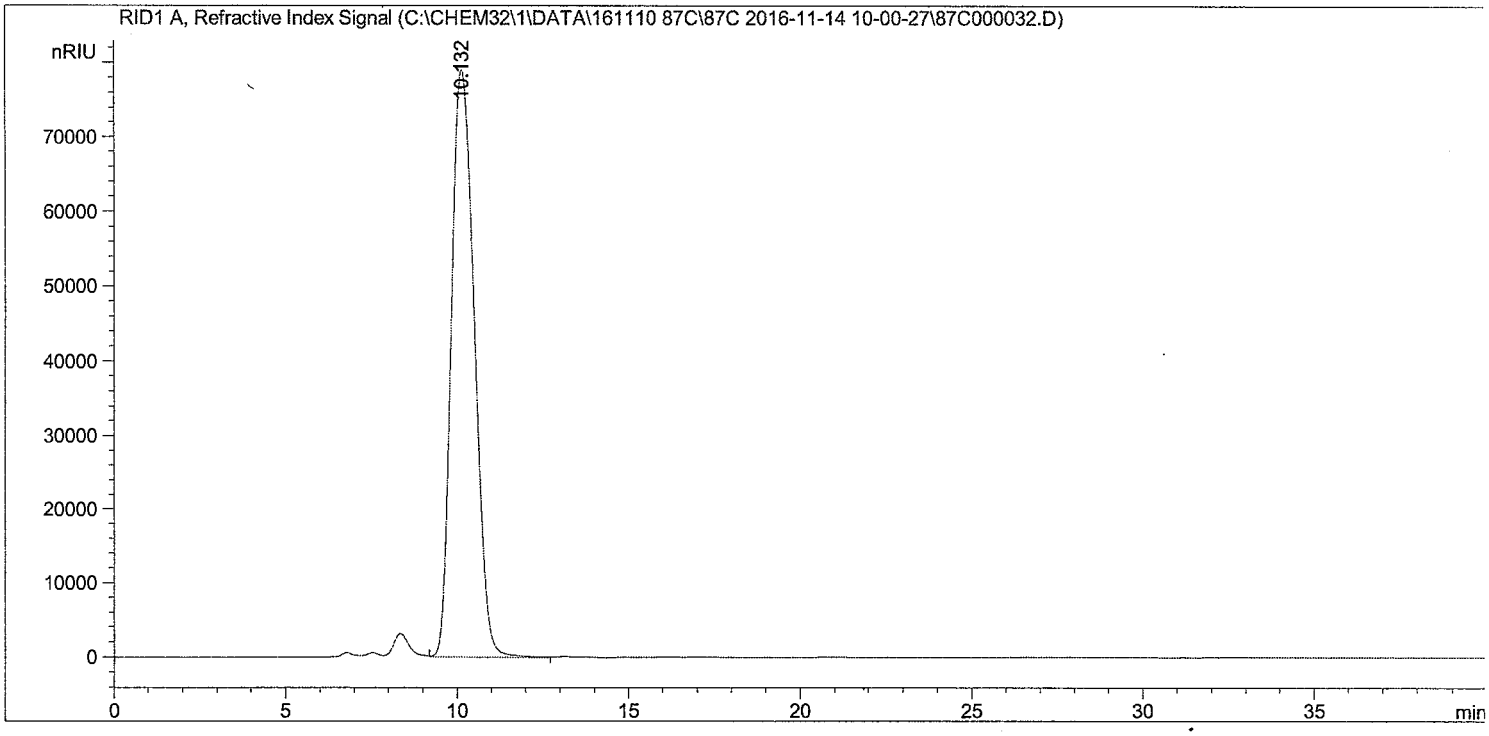
Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	7.021	VV	0.3121	1.36726e4	634.06299	0.0960
2	7.814	VV	0.3828	1.17499e4	462.50888	0.0825
3	8.673	VV	0.5975	5.13311e4	1354.96045	0.3604
4	9.630	VV F	0.3849	1.63428e4	603.86536	0.1148
5	10.520	VV	0.7380	5.10573e5	1.08712e4	3.5852

Data File C:\CHEM32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C000032.D
Sample Name: Glucose

==========

Acq. Operator : jinsol Seq. Line : 32
Acq. Instrument : Instrument 1 Location : Vial 8
Injection Date : 11/14/2016 11:18:36 PM Inj : 1
Inj Volume : 10 µl
Acq. Method : C:\Chem32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C-40MIN.M
Last changed : 1/21/2016 10:53:59 AM by Kwon sg
Analysis Method : C:\CHEM32\1\METHODS\87C-30MIN.M
Last changed : 11/15/2016 8:27:12 AM by Goeun
(modified after loading)



==========

Area Percent Report

==========

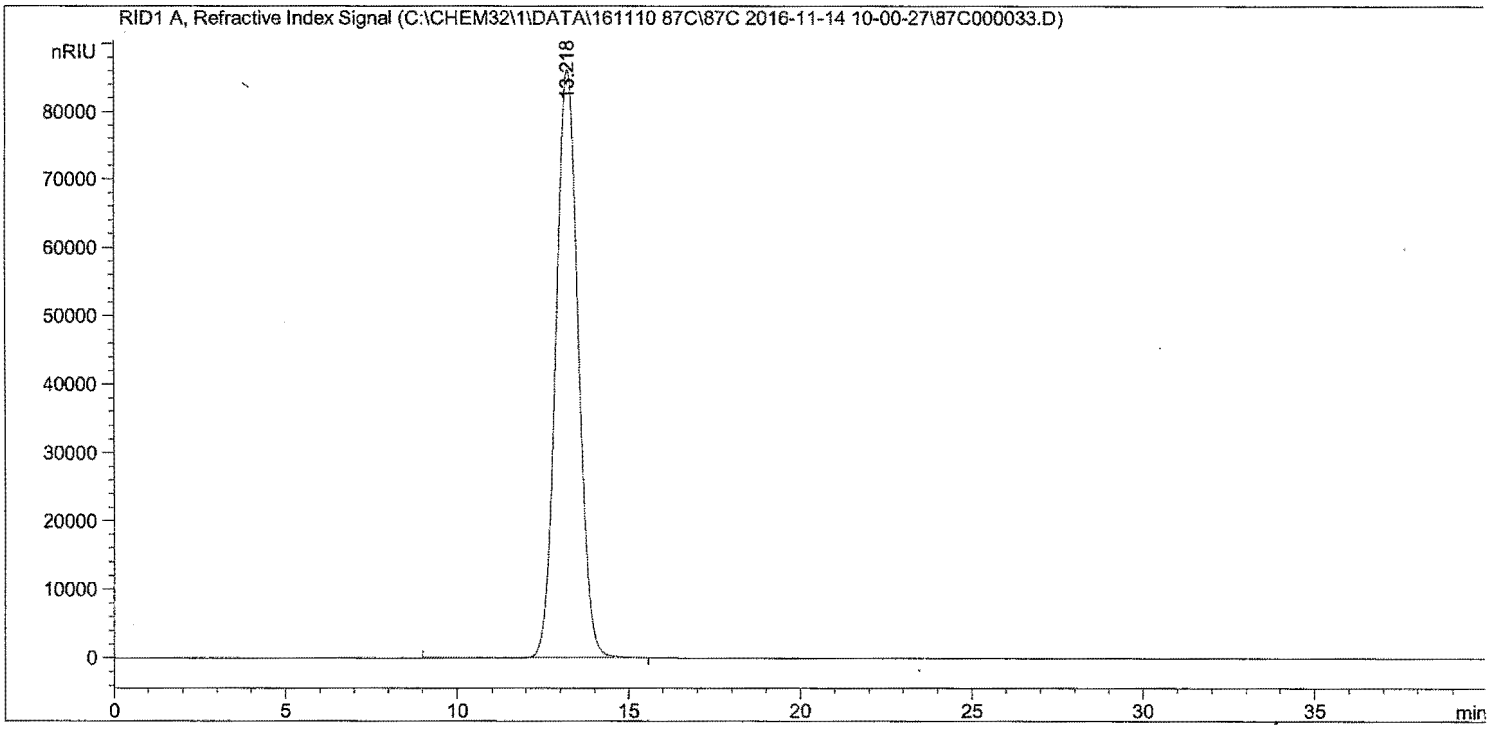
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	10.132	VV	0.7502	3.67952e6	7.90810e4	100.0000
Totals :				3.67952e6	7.90810e4	

Data File C:\CHEM32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C000033.D
Sample Name: Fructose

Acq. Operator	: jinsol	Seq. Line	: 33
Acq. Instrument	: Instrument 1	Location	: Vial 9
Injection Date	: 11/15/2016 12:00:10 AM	Inj	: 1
		Inj Volume	: 10 µl
Acq. Method	: C:\Chem32\1\DATA\161110 87C\87C 2016-11-14 10-00-27\87C-40MIN.M		
Last changed	: 1/21/2016 10:53:59 AM by Kwon sg		
Analysis Method	: C:\CHEM32\1\METHODS\87C-30MIN.M		
Last changed	: 11/15/2016 8:27:12 AM by Goeun		
	(modified after loading)		



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	13.218	VV	0.6997	3.89336e6	8.62145e4	100.0000
Totals :				3.89336e6	8.62145e4	

2.6. Composition and Specifications

As shown in Tables 1-1 to 1-4, the only differences in specification are found in the concentrations of D-allulose and moisture. Specifications for microbial and heavy metal content are the same for powder and liquid forms.

Table 1-1. Composition of Product 1=sum of macronutrients should be close to 100%. Please analyze for protein and fat. If there are other carbohydrates, please identify what those are like lactose, etc.

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	20~25	HPLC
D-fructose*	68~73	HPLC
D-glucose*	4~6	HPLC
Dextrin* (DS2~4)	1~3	HPLC
Protein	-	AOAC945.23
Fat	-	AOAC996.06
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis

D-Allulose + D-Fructose = 93%, D-glucose + Dextrin = 7%

Table 1-2. Composition of Product 2=sum of macronutrients should be close to 100%

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	50~55	HPLC
D-fructose*	40~45	HPLC
D-glucose*	1.5~4.0	HPLC
Dextrin* (DS2~4)	1.0~3.5	HPLC
Protein	-	AOAC945.23
Fat	-	AOAC996.06
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01

As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis

D-Allulose + D-Fructose = 95%, D-glucose + Dextrin = 5%

Table 1-3. Composition of Product 3=sum of macronutrients should be close to 100%. Please analyze for protein and fat

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥90	HPLC
Protein	-	AOAC945.23
Fat	-	AOAC996.06
Moisture	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis

Table 2-1. Specifications of Product 1 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥20	HPLC
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis

Table 2-2. Specifications of Product 2 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥50	HPLC
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis

Table 2-3. Specifications of Product 3 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥90	HPLC
Moisture	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis

Table 2-4. Specifications of Product 4 (Crystalline D-allulose, ≥98%)

Composition	Specification	Analytical Method
Appearance	Powder	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥98	HPLC
Moisture, %, wt/wt	≤2	AOAC 941.14
pH	3.0 – 7.0	pH meter

Ash, %, wt/wt	≤0.1	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming unit.

EXPERT PANEL REPORT

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

On behalf of SamYang Corp.

Prepared by: NutraSource, Inc.
6309 Morning Dew Court
Clarksville, MD 21029
Tel: 410-531-3336
Susanscho1@yahoo.com

GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-Allulose (D-psicose) AS A FOOD INGREDIENT

Table of contents

Tables

Figures

EXPERT PANEL REPORT

PART 1. CONCLUSION OF GENERALLY RECOGNIZED AS SAFE DETERMINATION FOR D-ALLULOSE (OR D-PSICOSE)

PART 1. EXECUTIVE SUMMARY AND EXPERT PANEL STATEMENT

We, the undersigned expert panel members, Susan Cho, Ph.D., George Fahey, Ph.D., and Joanne Slavin, Ph.D., have critically evaluated the safety of D-allulose (D-psicose).

On behalf of Samyang Corp., we, the undersigned expert panel members, Susan S. Cho, Ph.D., George Fahey, Ph.D., and Joanne Slavin, Ph.D., have independently evaluated the materials summarized in this GRAS report. Based on a critical evaluation of the publicly available data summarized herein, the Expert Panel members, whose signatures appear below, have individually and collectively, concluded that D-allulose, produced consistent with current Good Manufacturing Practices and meeting the specifications described herein, is safe under its intended conditions of use (as a nutritional food ingredient).

1.A. Common Knowledge Element of the GRAS Determination

D-allulose has been safely used as a food ingredient around the world for a decade. As a result, a number of comprehensive reviews of the safety of D-allulose have been published (Chung et al., 2012b). In addition, the FDA has had no question on two GRAS Notices related to safety of D-allulose (GRN 400, FDA 2012; GRN 498, FDA, 2014).

1.B. Technical Element of the GRAS Determination (Safety Determination)

Numerous human and animal studies have reported benefits of D-allulose with no major adverse effects. Samyang Corp.'s D-allulose is manufactured under cGMP using common food industry materials and processes. Samyang Corp. uses a 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 concerning the chemistry of D-allulose. This GRAS determination is based on the data and information generally available and consented opinion about the safety of D-allulose. The literature indicates that D-allulose offers consumers benefits without adverse effects.

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 and meet all applicable purity standards.
2. Samyang Corp.'s D-allulose will be used as a sugar substitute in food applications at use levels ranging from 2 to 100%: selected (low or reduced calorie) bakery products, beverages, cereals, chewing gums, confections and frostings, frozen dairy desserts, yogurt and frozen yogurt, dressings for salads, gelatins, puddings and fillings, hard and soft candies, jams and jellies, sugar, sugar substitutes, sweet sauces and syrups, and fat-based creams.
3. The LD_{50} value of D-allulose in rats has been reported as 15.8-16.3 g/kg. A chronic toxicity study 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 3% of the diet, the highest level tested.

4. A human clinical study showed that the maximum tolerable levels in humans were 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females. The only side effect of non-digestible carbohydrates including D-allulose is gastrointestinal discomfort when ingested in large quantities. This type of symptom is usually transient and is not considered to be of toxicological significance (IOM, 2002).
5. The proposed food use results in exposure at levels below those associated with any adverse effects. The EDI estimates are based on the assumption that Samyang Corp.'s D-allulose will replace currently marketed D-allulose. Thus, cumulative exposures are not expected. In addition, the EDIs presented in this notice are highly optimistic estimates.
6. In the previous GRAS notices (GRN 400 and 498) to the FDA, the safety of D-allulose has been established in animal toxicity studies and mutagenicity studies, and is further supported by human clinical studies.
7. Additional animal studies published subsequent to the FDA GRAS notices continue to support the safety of D-allulose as a food ingredient.

Overall, there are no indications of significant adverse effects related to D-allulose in the publicly available literature. 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).

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have concluded that D-allulose, when used as described in this dossier, is GRAS based on scientific procedures.

(b) (6)

Susan Cho, Ph.D.
NutraSource, Inc., Clarksville, MD 21029

1/22/2017
Date

(b) (6)

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

1/20/17
Date

(b) (6)

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

12-29-16
Date

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

A. Scientific Information About the Identity of a Notified Substance

2.A.1. Scientific Information Sufficient To Identify a Biological Source

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

Standards of Identity

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

Chemistry, Physicochemical Properties, and Structure

Chemical name is D-ribo-2-ketohexose
MW=180.16
Molecular formula: $C_6H_{12}O_6$
CAS Registry ID; 551-68-8

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

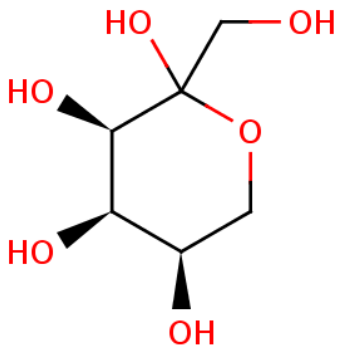


Figure 1. Chemical Structure of D-allulose

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

No toxicant production is expected in the manufacture of allulose. The final product is highly purified through several steps during production. Further, the enzymatic conversion of D-fructose to D-allulose is an enzymatic reaction that occurs in nature, with no known toxicant production.

2.A.3. Particle Size

NLT 90% pass 40 mesh.

2.B. Method of Manufacture

D-allulose is manufactured from fructose in aqueous solution by enzymatic epimerization in the presence of magnesium chloride. The enzyme used is an immobilized D-allulose-3-epimerase, which converts fructose to D-allulose. Compared to those described in previous GRAS notices, SamYang Corp. employs a unique immobilized enzyme system described below. The enzyme system has been proven safe.

Differences in enzyme systems described in various GRNs

Current notice - SamYang Corp.

The neutralized fructose syrup is passed into an immobilized cell system (calcium alginate gel bead with recombinant *Corynebacterium glutamicum* [non-viable cell] harboring D-allulose 3-epimerase [DPE] from *Clostridium scindens*). The fructose then is converted to D-allulose at 50℃.

GRN 400 - CJ CheilJedang

An immobilized cell system (calcium alginate gel bead with *Corynebacterium glutamicum* [non-viable cell] harboring D-psicose 3-epimerase [DPE] originated from *Agrobacterium tumefaciens).*

GRN 498 - Matsutani

D-psicose 3-epimerase (DPE) is extracted from *Escherichia coli* (K12) [non-viable cell] or *Streptomyces violaceoruber* harboring DPE that originated from *Arthrobacter globiformis or Arthrobacter globiformis* itself.

SamYang's Manufacturing process

1. The fructose syrup (≥75% solids concentration) is diluted with clean water (>50% solids concentration) in a reception tank and then stored in a stock tank.
2. The neutralized fructose syrup is passed into an immobilized cell system (calcium alginate gel bead with recombinant *Corynebacterium glutamicum* [non-viable cell] harboring D-allulose 3-epimerase [DPE] from *Clostridium scindens*). The fructose then is converted to D-allulose at 50℃.
3. For decolorization and desalting, the D-allulose solution is mixed with active carbon in a stirred tank reactor. The liquid undergoes pressure filtration to clarify it, and it is treated through an ion exchange process (i.e., a cation column with strongly acidic cationic exchange resin; an anion column with intermediate basic anion exchange resin; and a mixed bed column that has a combination of both strongly acidic and strongly basic resins) to remove any impurities (e.g. calcium, manganese, chloride, and other ionic components, including amino acids, peptides, and proteins).
4. Following ion exchange purification, the D-allulose solution is concentrated with an evaporator to produce syrup (Product 1-Allulose syrup, ≥20% on a dry weight basis).
5. This concentrated syrup is pumped into a separation chromatography system to separate D-allulose from other sugars (i.e., fructose).
6. Using an evaporator, the solution is concentrated to the final density of ≥65 ºBx to produce syrup (Product 2 or 3- D-allulose syrup, ≥50% or ≥90% on a dry weight basis).

7. The final concentrated product is pumped into a batch continuous crystallizer.
8. The crystalline D-allulose (Product 4 - ≥98% D-allulose) is separated by basket centrifugation, washed by spraying distilled water, and finally dried in a rotary dryer.

Quality assurance procedure:

Samyang Corp.'s D-allulose is manufactured under current Good Manufacturing Practices (cGMP) using common food industry materials and processes. Samyang Corp. 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 grades. D-allulose is manufactured under cGMP using common food industry materials and processes in accordance with the applicable parts of 21 CFR, part 110 of the Code of Federal Regulations. Process tanks and lines are cleaned with sodium hydroxide and hydrogen peroxide following standard procedures common to the dairy industry. 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 2.

Safety of enzymes:

The enzyme utilized is non-toxicological and non-pathogenic. An acute toxicity study showed that a single dose of 2 g/kg bw did not cause any treatment-related abnormalities in Sprague-Dawley rats. The LD_{50} was determined to be far above 2 g/kg bw.

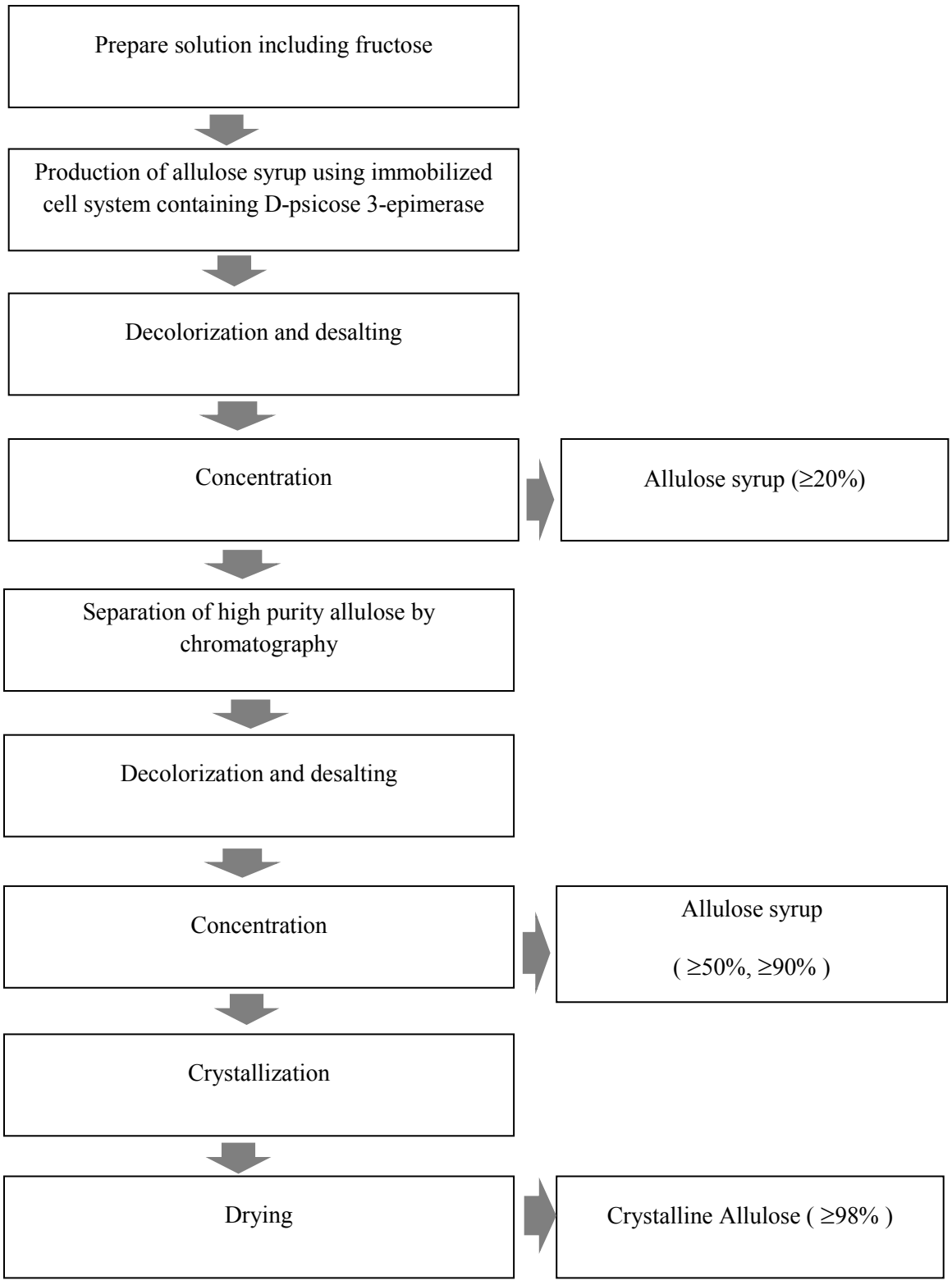


Figure 2. Flow Diagram of Manufacturing Process

2.C. Specifications of D-allulose

As shown in Tables 1-1 to 1-3 and 2-1 to 2-4, the only differences in composition and specification are found in the concentrations of D-allulose, excipients (glucose, fructose and dextrin) and moisture. Specifications for microbial and heavy metal content are the same for powder and liquid forms.

Table 1-1. Composition of Product 1

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	20~25	HPLC
D-fructose*	68~73	HPLC
D-glucose*	4~6	HPLC
Dextrin* (DS2~4)	1~3	HPLC
Protein	ND	AOAC 945.23
Fat	ND	AOAC 996.06
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; D-Allulose + D-Fructose = 93%; D-glucose + Dextrin = 7%; CFU=colony forming units; ND=not detected.

Table 1-2. Composition of Product 2

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	50~55	HPLC
D-fructose*	40~45	HPLC
D-glucose*	1.5~4.0	HPLC
Dextrin* (DS2~4)	1.0~3.5	HPLC
Protein	-	AOAC 945.23
Fat	-	AOAC 996.06
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter

Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; D-Allulose + D-Fructose = 95%; D-glucose + Dextrin = 5%; CFU=colony forming units; ND=not detected.

Table 1-3. Composition of Product 3

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥90	HPLC
Protein	ND	AOAC945.23
Fat	ND	AOAC996.06
Moisture	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units; ND=not detected.

Table 2-1. Specifications of Product 1 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥20	HPLC
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01

Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

Table 2-2. Specifications of Product 2 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥50	HPLC
Moisture, %, wt/wt	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

Table 2-3. Specifications of Product 3 (D-allulose Syrup)

Composition	Specification	Analytical Method
Appearance	Clear yellow liquid	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥90	HPLC
Moisture	≤35	AOAC 941.14
Brix	≥65	Brix meter
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.5	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

Table 2-4. Specifications of Product 4 (Crystalline D-allulose, ≥98%)

Composition	Specification	Analytical Method
Appearance	Powder	Visual
Odor	No odor	
D-allulose*, %, wt/wt	≥98	HPLC
Moisture, %, wt/wt	≤2	AOAC 941.14
pH	3.0 – 7.0	pH meter
Ash, %, wt/wt	≤0.1	AOAC 900.02
Pb, ppm	≤0.5	AOAC 2015.01
As, ppm	≤0.5	AOAC 2015.01
Cd, ppm	≤0.5	AOAC 2015.01
Total plate count, CFU/g	≤1,000	AOAC 2002.07
Coliforms	negative	AOAC 991.14
Salmonella	negative	AOAC 989.14
Staphylococcus aureus	negative	AOAC 987.09

*Dry wt. basis; CFU=colony forming units.

PART 3. DIETARY EXPOSURE

3.A. Food Sources of D-allulose

As shown in Table 3, D-allulose is a naturally occurring monosaccharide present in small quantities in food products, particularly in selected bakery products, sweets, and fruits (Oshima et al., 2006).

Table 3. D-allulose content in foods (adopted from Oshima et al., 2006)

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
Raisin	38.7
Canned peaches	1.5
Can of mandarin oranges	8.4
Canned cherries	2.0

3.B. Intended Use

Intended use and use levels of Samyang Corp.'s D-allulose have been adopted from GRN 498 and GRN 400. SamYang Corp. proposes to use D-allulose as a sugar substitute in food applications at use levels ranging from 2 to 100%. As shown in Table 4, intended applications include: bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics), beverages (non-alcoholic, low or reduced calorie, sugar free); cereals; chewing gums; confections and frostings; frozen dairy desserts (ice cream, soft serve, sorbet) (low calorie, reduced calorie, sugar-free); yogurt and frozen yogurt (low calorie, reduced calorie, sugar-free); dressings for salads; gelatins, pudding and fillings (low calorie, reduced calorie, sugar-free); gelatins, pudding and fillings (low calorie, reduced calorie, sugar-free); hard and soft candies (low calorie, reduced calorie, sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low calorie, reduced calorie, sugar-free) and fat based cream. Please note that intended use and use levels are mostly adopted from GRN 498 which completely replaced sugars (100%) in some bakery products (GRN 498 listed some bakery products in the sugar category and that GRN 400 used up to 10% D-allulose in some bakery products. Thus, we have mostly adopted the intended use and use levels from GRN 498 and have added the food category which is not included in GRN 498, but in GRN 400.

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

Table 4. Intended Use and Maximum Use Levels of D-allulose, % (w/w)

Food category	Maximum use levels, % (w/w)
Bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics)	10-100
Beverages (non-alcoholic), low calorie, reduced calorie, sugar-free	3.5
Cereals, regular Cereals, low calorie, reduced calorie, sugar-free	2 5
Chewing gum	50
Confections and frostings	5
Frozen dairy desserts (ice cream, soft serve, sorbet), low calorie, reduced calorie, sugar-free	5
Yogurt and frozen yogurt, low calorie, reduced calorie, sugar-free	5
Dressings for salads	5
Gelatins, pudding and fillings, low calorie, reduced calorie, sugar-free	10
Hard candies, low calorie, reduced calorie, sugar-free	50
Soft candies, low calorie, reduced calorie, sugar-free	25
Jams and jellies	10
Sugar	10
Sugar substitutes	100
Sweet sauces and syrups, low calorie, reduced calorie, sugar-free	10
Fat-based cream (used in modified fat/calorie cookies, cakes, pastries, and pie)	5

3.C. Estimated Daily Intakes (EDIs) of Naturally Occurring D-allulose from the Diet

The D-allulose level in each food is not listed in the USDA food composition tables or the National Health and Nutrition Examination Survey (NHANES) databases. Using the dietary content of D-allulose available from the studies of Oshima et al. (2006; Table 3), the EDIs from the diet were estimated. The mean and 90th percentile EDIs of users are 94.8 and 260.7 mg D-allulose/person/day. 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.

Table 5-1. Intake of Naturally Occurring Allulose from the Diet (all users)

Age, y	N	mg/person/day				mg/kg bw/day				Body wt., kg	
		Mean	SE	P 90	SE	Mean	SE	P 90	SE	Mean	SE
All gender											
1-99 y	8126	94.8	2.5	260.7	12.2	1.46	0.04	3.97	0.12	72.0	0.4
1-6 y	1155	47.0	2.3	117.1	10.7	2.86	0.16	6.93	0.55	17.6	0.2
7-12 y	1074	55.2	3.3	141.0	3.4	1.54	0.09	3.66	0.26	40.5	0.8
13-19 y	1009	99.8	6.7	271.6	10.8	1.53	0.11	4.36	0.30	67.7	1.2
20+ y	4800	104.0	3.0	283.2	11.7	1.28	0.04	3.52	0.16	81.9	0.5
Males											
13-19 y	514	103.8	11.0	284.0	16.6	1.53	0.15	4.44	0.39	72.5	1.2
20+ y	2393	120.7	6.0	295.8	22.5	1.39	0.07	3.89	0.20	88.3	0.6
Females											
13-19 y	495	95.2	14.3	225.2	34.7	1.52	0.22	4.06	0.77	62.5	1.5
20+ y	2407	88.2	3.8	258.9	14.8	1.18	0.04	3.26	0.17	75.8	0.6

BW=body weight; P90=90th percentile; Based on NHANES 2011-2014.

Table 5-2. Intake of Naturally Occurring Allulose from the Diet (total population)

Age, y	N	mg/person/day				mg/kg bw/day				Body wt., kg	
		Mean	SE	P 90	SE	Mean	SE	P 90	SE	Mean	SE
All gender											
1-99 y	8126	84.5	2.3	233.8	14.9	1.30	0.04	3.69	0.15	72.0	0.4
1-6 y	1243	44.4	2.2	116.31	10.8	2.71	0.15	6.89	0.56	17.6	0.2
7-12 y	1074	48.8	3.0	136.0	4.8	1.36	0.08	3.45	0.13	40.5	0.8
13-19 y	1009	82.6	9.4	245.5	19.8	1.27	0.12	3.89	0.30	67.7	1.2
20+ y	4800	92.9	2.8	274.2	12.2	1.15	0.03	3.30	0.17	81.9	0.5
Males											
13-19 y	514	89.9	9.8	280.0	13.9	1.33	0.13	4.40	0.48	72.5	1.2
20+ y	2393	107.5	5.1	285.4	17.0	1.24	0.06	3.59	0.24	88.3	0.6
Females											
13-19 y	495	74.9	12.4	198.6	22.4	1.20	0.21	3.39	0.79	62.5	1.5
20+ y	2407	79.1	3.5	216.1	13.6	1.06	0.04	3.00	0.13	75.8	0.6

BW=body weight; P90=90th percentile; Based on NHANES 2011-2014.

3.D. Exposure Estimates Under the Intended Use

3.D.1. EDI of D-allulose Under the Intended Use

The intended use of D-allulose is in the same food products and at levels proportional to those mentioned in the GRN 498 and GRN 400. The results of the EDI assessment are summarized in the two tables below. The first table presents the results of the mean of the population as well as the 90th percentile in g/day, and the second in g/kg bw/day. Since intended use and use levels combined those described in GRN 498 and 400, the EDIs in this GRAS determination are estimated to be slightly higher than those described in the two GRAS notices. However, EDIs presented in this GRAS notice are within the safe intake levels. These results reveal an average maximum exposure would occur in males greater than 19 years of age, with a 90th percentile value of 36.3 g/day or 0.39 g/kg bw/day. On a body weight basis, children aged 2-12 years had shown the highest 90^{th} percentile EDI at 0.50 g/kg bw/day. All subpopulation groups had the EDIs below 0.5 g/kg bw/day. The toxicity data reveals an LD50 of 15.8-16.3 g/kg bw, indicating that even at the highest exposure, D-allulose is not a safety risk.

These estimates are highly optimistic since it is not likely that D-allulose will be used at maximum levels for all food categories under the intended uses. Also, food wastes should be considered. Overall, intended use will result in EDIs at levels significantly below those associated with any potential side effects.

Table 6-1. Maximum EDIs of D-allulose, g/day * (assuming all the foods will be used at the maximum use levels)

		Per User (g/day)		Per Capita (g/day)	
Population	N-user*	Mean	90^{th} Percentile	Mean	90^{th} Percentile
US 2+ y	13,455	11.0	30.0	8.6	24.8
Infants < 2 y	536	0.8	2.6	1.7	4.1
Children 2-12 y	3,223	5.2	14.2	4.1	12.0
Adolescents 13-18 y	1,283	7.6	16.7	5.1	14.6
Males 19+ y	4,178	13.0	36.3	9.8	29.0
Females 19+ y	4,771	12.7	32.6	10.0	29.3

* Based on NHANES 2007-10.

Table 6-2. Maximum EDIs of D-allulose, g/kg bw/day (assuming all the foods will be used at the maximum use levels) NHANES 2007-10

Population	N-user*	Per User (g/kg bw/day)		Per Capita (g/kg bw/day)	
		Mean	90th Percentile	Mean	90th Percentile
US 2+ y	13,455	0.16	0.42	0.12	0.35
Infants < 2 y	536	0.08	0.24	0.15	0.42
Children 2-12 y	3,223	0.19	0.50	0.15	0.42
Adolescents 13-18 y	1,283	0.12	0.29	0.08	0.24
Males 19+ y	4,178	0.14	0.39	0.11	0.31
Females 19+ y	4,771	0.16	0.44	0.13	0.38

* Based on NHANES 2007-2010. BW=body weight.

3.D.2. EDI of Other Components Under the Intended Use

Two D-allulose syrup products (Products 1 and 2) contain other nutrients such as fructose and glucose. Glucose is subjected to 21CFR 184.1277and 168.120. Fructose (in the form of high fructose corn syrup) is subjected to 21CFR 184.1866. Thus, we have not calculated the EDIs of these nutrients from the diet.

PART 4. SELF-LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with the D-allulose ingredient.

PART 5. THE 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.

PART 6. BASIS FOR OUR CONCLUSION OF GRAS STATUS

6.A. Current Regulatory Status

The FDA has received two GRAS notices related to food uses of D-allulose (GRN 400 submitted by CJ CheilJedang, Inc., 2011; GRN 498 submitted by Matsutani Chemical, 2014). In these GRAS notices, toxicity-related studies on D-allulose from the literature were presented that support the safety of use of D-allulose. The FDA did not question the acceptability and suitability of these studies to establish the safety of D-allulose for the proposed food uses. The FDA did not have questions on the summary of safety, concluding that D-allulose intake of less than 0.5 g/kg bw/day is safe. Table 4 summarizes previous GRAS notices and the current notice for D-allulose.

Table 7. Summary of Previous and the Current GRAS Notices

GRN	Company	Intended use	EDI, 90th pctl for all users
400	CJ CehilJedang	As a sugar substitute in dietetic or low calorie bakery products, chewing gums, fat-based cream used in modified fat/calorie cookies, cakes and pastries, low calorie hard candies including pressed candy and mints, low calorie frozen dairy desserts, low calorie carbonated beverages, reduced and low calorie non-carbonated beverages, sugar substitutes, low calorie yogurt, medical foods, ready-to-eat cereals (<5% sugar), and coffee mix.	28.5 g/person/day or 0.36 g/kg bw/day
498	Matsutani	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	24.8 g/person/day (0.33 g/kg bw/day
Present notice	Samyang Corp.	As a sugar substitute in food applications at use levels ranging from 2 to 100%.	30 g/person/day or 0.42 g/kg bw/day

bw= body weight; GRAS= generally recognized as safe; pctl=percentile.

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.

6.B. Intended Technical Effects

D-allulose will be used as a food ingredient for low calorie and/or dietetic foods due to its technological properties (e.g., functions as a sweetener, humectant, and flavor modifier) and nutritional benefits (such as low calorie and glycemic control).

6.C. Review of Safety Data

As noted above, the FDA has had no question on two GRAS notices related to food uses of D-allulose. The FDA did not have questions on the summary of safety concluding that D-allulose intake up to 0.5 - 0.6 g/kg bw/day is safe. Since the specifications for the liquid and powder forms of D-allulose in this notice are similar to those described in GRN 400 and 498, the metabolism and safety data and other pertinent information discussed in GRN 400 and 498 are applicable to the safety of D-allulose in this GRAS notice. The information is hereby incorporated by reference in these documents and will not be discussed in detail.

Since the FDA's review of GRNs 400 and 498 (GRN 400, FDA, 2012; GRN 498, FDA, 2014), five animal studies were published; one metabolism (Tsukamoto et al., 2014) and four efficacy studies (Hossaine et al., 2015; Itoh et al., 2015; Nagata et al., 2015; Ochiai et al., 2014). Findings from these studies were not inconsistent with the agency's prior decision.

6.C.1. Metabolism

A study published since the FDA's decision of 2014 confirmed the previous findings that D-allulose was rapidly excreted through urine (Tsukamoto et al., 2014). Following oral administration, D-allulose is partly absorbed in the digestive tract and enters the bloodstream. The maximum blood concentration (48.5±15.6 μg/g) was observed at 1 hour. Excretion via urine was 20% within 1 hour and 33% within 2 hours (Tsukamoto et al., 2014). Accumulation in organs was detected only in the liver. Following intravenous administration, blood concentration of D-allulose was decreased with the half-life of 57 minutes, and the excretion via urine reached almost 50% within 1 hour. Seven days after the single-dose oral administration, the remaining amounts in the whole body was less than 1%.

Previously reviewed studies reported that about 98% of intravenously administered D-allulose is excreted in the urine within 6 h (Whistler et al., 1974). When orally ingested, urinary excretion of unchanged D-allulose ranged from 11 to 25% (Matsuo et al., 2003). The data indicate that D-allulose 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-allulose is fermented to short chain fatty acids (SCFA) by intestinal microflora in the colon (Noda and Oh, 1992) or is excreted in the feces (Matsuo et al., 2004).

6.C.2. Animal Toxicity Studies

Since the FDA's last review of D-allulose in 2012-2014 (GRNs 400 and 498; U.S. FDA, 2012 and 2014, respectively), one new paper has been published (Nishi et al., 2016a). This study reported that a single oral dose of 1 or 4 g/kg bw did not cause any treatment-related abnormalities in dogs. All dogs were active and had a good appetite throughout the study period. Blood glucose concentration slightly decreased without a rise in plasma insulin concentration 2 h after D-allulose administration. Plasma alkaline phosphatase activities showed a mild increase between 12 and 48 h after D-allulose administration. These data suggested that a single oral dose of D-allulose does not show severe toxicity in dogs.

Previous reviews included the LD_{50} value of D-allulose in rats at 15.8-16.3 g/kg bw (Matsuo et al., 2002). Subacute toxicity studies (up to 34 days) in rats showed that D-allulose concentration of up to 20% of the diet did not show adverse effects (Table 4; Matsuo et al., 2002). A 90 day

subchronic toxicity study reported the no observed adverse effect level (NOAEL) for D-allulose as 3% of diet, the highest level tested (Matsuo et al., 2012). A 12-18 month chronic toxicity study showed that D-allulose at the dose of 3% D-allulose in the diet (or 1,280 mg/kg bw/day), the highest level tested, did not show adverse effects (Yagi and Matsuo, 2009).

In summary, D-allulose, like other monosaccharides, belongs to the group that has the lowest toxicity rating and is classified as an ordinary carbohydrate substance. Thus, the use of D-allulose in foods and beverages is not expected to pose a safety concern.

Table 8. Summary of Animal Toxicity Studies Referenced in GRNs 400 and 498

Species	Dosage	Duration	Primary endpoints and NOAEL	Reference
Dogs	1 and 4 g/kg bw	Single dose	Acute toxicity-food intake and selected clinical chemistry	Nishi et al., 2016a
Male rats	8, 11, 14, 17, and 20 g/kg bw (D-allulose in water)	Single dose	Acute toxicity-LD_{50}, 16.3 g/kg bw	Matsuo et al., 2002
Young rats	10, 20, 30, and 40% in the diet	34 days	Feed intake, wt. gain, and organ wt.; NOAEL-up to 20% in the diet (corresponding to 10,000 mg/kg bw/day)	Matsuo et al., 2002
Male Wistar rats	3% in the diet	90 days	Feed intake, wt gain, organ wt., serum biochemistry, hematology, and histology; NOAEL- 3% in diet, the highest level tested	Matsuo et al., 2012
36 Male rats, Wistar	3% in the diet or 1,280 mg/kg bw/d (control, 3% sucrose)	12-18 months	Feed and energy intakes, wt. gain, organ wt., digestive tract size, serum biochemistry, hematology, and histology; NOAEL- 1,280 mg/kg bw/day, the highest level tested	Yagi and Matsuo, 2009

bw= body weight; NOAEL= no observed adverse effect level; wt= weight.

6.C.3. Animal Efficacy Studies Reporting No Adverse Effects of D-allulose

Since the FDA's last review of D-allulose (GRNs 400 and 498; U.S. FDA, 2012 and 2014, respectively), four animal efficacy studies were published based on the repeat dose administration of D-allulose at high dietary concentrations for long durations (Table 6; Han et al., 2016; Hossain et al., 2015; Itoh et al., 2015; Nagata et al., 2015; Nishi et al., 2016b; Ochiai et al., 2014). No studies reported results inconsistent with the FDA's prior reviews of 2012-2014. Although these studies were designed to investigate the efficacy of D-allulose on various health parameters, several safety-related endpoints were obtained during the experiments. Therefore, these studies are reviewed below as additional supporting information.

Recent efficacy studies showed that D-allulose at the level of up to 5% in the diet (corresponding to up to 2,500 mg/kg bw/day) did not cause any adverse effects on food efficiency, glucose metabolism, lipid metabolism, inflammatory biomarkers, body fat accumulation, and/or histopatholgical parameters (Han et al., 2016; Hossain et al., 2015; Itoh et al., 2015; Nagata et al., 2015; Nishi et al., 2016b; Ochiai et al., 2014).

Nishi et al. (2016b) reported that oral administration of D-allulose (0.2 g/kg bw) decreased plasma glucose concentrations after oral glucose or maltose administration, with a diminished plasma insulin rise in dogs. However, D-allulose showed no effect on plasma glucose and insulin concentrations after feeding. The data suggest that D-allulose administration may be beneficial in dogs with impaired glucose tolerance.

In a study by Han et al. (2016), mice were fed a high fat diet with or without various sugar substitutes (d-glucose, d-fructose, erytritol, or D-allulose, n = 10 per group) for 16 wk. Body weight and fat-pad mass in the D-allulose group were dramatically lowered to that of the normal group with a simultaneous decrease in plasma leptin and resistin concentrations. d-allulose lowered plasma and hepatic lipids while elevating fecal lipids. In the liver, activities of both fatty acid synthase and β-oxidation were downregulated by D-allulose to that of the normal group; however, in WAT, fatty acid synthase was decreased while β-oxidation activity was enhanced. No adverse effects of D-allulose were reported.

Long-term administration (60 weeks) of D-allulose at a dose of 5% of the diet prevented the commencement and progression of type 2 diabetes through the maintenance of blood glucose levels and the control of postprandial hyperglycemia with decreased levels of HbA_{1c} (by ~50%) in comparison to control rats (Hossaine et al., 2015). This improvement in glycemic control was accompanied by the maintenance of plasma insulin levels and the preservation of pancreatic β-cells with a significant reduction in inflammatory markers. In the control group, the glucose levels started to increase slowly from 25 weeks and then sharply until 60 weeks, whereas in the allulose group the glucose levels started to increase slightly from 45 weeks and remained constant until 60 weeks. By the end of 60 weeks, the fasting blood glucose concentrations in the psicose group were approximately 35% lower than that of the control group. Body fat accumulation, in particular adipose tissue, was lower (by ~25-30%) in the treatment group, with decreased infiltration of macrophages in the abdominal adipose tissue. No adverse effects of D-allulose were reported.

The study by Itoh et al. (2015) also reported anti-obesity effects of D-allulose (0, 2.5, or 5% of the diet or 1,500-2,000 or 3,000-4,000 mg/kg bw/day) in inherited leptin-deficient ob/ob mice. Wild type C57BL/6J mice were used as an animal control (0% D-allulose). The results of this study showed that subchronic ingestion for 15 weeks significantly decreased body weights (by ~20%), liver weights (by ~6%), and total fat mass (by ~7%), including abdominal visceral fat (by ~5%) in the 5% allulose group. During the 15-week period, the total calorie intake of the 5% D-allulose treatment significantly decreased by 10% compared to that observed in both the control and 2.5% D-allulose groups. Furthermore, D-allulose improved hepatic steatosis as evaluated using hepatic histological evaluation and magnetic resonance imaging (MRI). In control mice, fat deposition produced a severely damaged liver histology presenting as remarkable ballooning degeneration. The ballooning degeneration and hepatic steatosis improved after the subchronic ingestion of D-allulose. The authors concluded that D-allulose may be useful

as a supplement for preventing and improving obesity and obesity-related disorders. No adverse effects of D-allulose were reported.

In a study by Nagata et al. (2015), effects of D-allulose on lipid metabolism were evaluated. Rats were fed diets with or without 3% D-allulose for 4 weeks. In experiment 1, feeding D-allulose significantly decreased body weight by approximately 5%, but not food intake. Liver enzyme activities involved in lipogenesis were significantly lowered by the D-allulose diet, whereas gene expression of a transcriptional modulator of fatty acid oxidation was enhanced. Rats fed D-allulose had significantly lower serum insulin and leptin levels. In experiment 2, feeding the D-allulose diet resulted in significantly lower body weight (389±3 vs. 426±6 g, $p < 0.05$) and food intake (23.8±0.2 vs. 25.7±0.4 g/day, $p < 0.05$) compared to the control diet. Rats fed the D-allulose diet had significantly higher energy expenditure in the light period and fat oxidation in the dark period compared to rats fed the control diet, whereas carbohydrate oxidation was lower. The results indicate that the D-allulose diet decreased lipogenesis, increased fatty acid oxidation, and enhanced 24 h energy expenditure, leading to D-allulose's potential for weight management. No adverse effects of D-allulose were reported.

These studies confirmed the previous findings that D-allulose at the level of up to 5% in the diet did not cause treatment-related abnormalities on measured outcomes (Table 6; Baek et al., 2010; Chung et al., 2012a; Hossain et al., 2012; Matsuo et al., 2001a, 2001b; Matsuo and Izumori, 2004, 2006, 2009; Ochiai et al., 2013).

Animal efficacy studies are summarized in Table 9. None of the animal efficacy studies reported adverse effects of D-allulose. For these 'pivotal' studies, the dose levels represent the maximum doses administered, rather than absolute safety endpoints.

Table 9. Animal Efficacy Studies Reporting No Adverse Effects of D-allulose

Species	Dosage	Length	Primary endpoints	Reference
Recent Animal Efficacy Studies				
Dogs	0.2 g/kg bw	Single dose	Blood glucose and insulin parameters	Nishi et al., 2016b
Mice	5% of high fat diet		Body weight, plasma concentrations of leptin and resistin, plasma and hepatic levels of lipids, and fecal excretion of lipids	Han et al., 2016
Young male Wistar rats	5% of high sucrose diet or control diet	8 weeks	Feed intake, wt. gain, clinical chemistry, energy expenditure, and body fat accumulation	Ochiai et al., 2014
Diabetic rats	5% of diet	60 weeks	Body weight gain, glucose metabolism, inflammatory biomarkers, and abdominal fat deposition.	Hossain et al., 2015
Rat, Sprague Dawley	3% of diet	4 weeks	Lipid metabolism (serum and liver lipid levels, liver enzyme activity, and gene expression)	Nagata et al., 2015

Mice (ob/ob and wild type C57BL/6J)	0, 2.5, or 5% of diet	15 weeks	Body and fat weights, liver weights, and hepatic steatosis	Itoh et al., 2015
Studies Referenced in GRNs 400 and 498				
Rat, Sprague-Dawley	5% of high fat diet	8 weeks	Feed intake, wt. gain, liver wt., visceral fat mass, blood lipid profile	Chung et al., 2012a
Male Wistar rats	5% of high sucrose diet or high starch diet	8 weeks	Body weight, food intakes, organ wt., serum clinical chemistry, liver triglycerides, carbohydrates and glycogen, and body fat	Ochiai et al., 2013
Diabetic rats	5% of diet	13 weeks	Body weight, glucose metabolism, inflammatory biomarkers, and abdominal fat deposition.	Hossain et al., 2012
Male mice	0.2 g/kg bw/d	4 weeks	Glycemic responses, insulin release, and blood lipid profiles, 0.2 g/kg bw/day	Baek et al., 2010
24 Male rats, Wistar	5% in the high (25%) and low fat (5%) diets	16 weeks	Body weight, energy intake, body fat, organ wt., glucose tolerance, serum adipocytokine concentrations (adiponectin, tumor necrosis factor alpha, leptin), and liver glycogen and triglycerides.	Matsuo and Izumori, 2004
Male rat	5% in the diet	3 weeks	Body fat and lipid metabolism	Matsuo et al., 2001a
Male rat	5% in the diet	4 weeks	Body fat and lipid metabolism	Matsuo et al., 2001b
Male rat	5% in the diet	8 weeks	Body fat and glycemic responses	Matsuo and Izumori, 2006
Male rat	2,000 mg/kg bw	Single dose	Body fat and glycemic responses	Matsuo and Izumori, 2009

bw= body weight; d= day

6.C.4. Human Clinical Studies

Since the FDA's last review of D-allulose in 2014 (GRNs 400 and 498; U.S. FDA, 2012 and 2014, respectively), no new literature has been published. Several human clinical studies previously reviewed reported no adverse effects of D-allulose (Table 6; Hayashi et al., 2010; Iida et al., 2007, 2008, 2010). Like non-digestible oligosaccharides and fiber ingredients, the only known side effect of D-allulose is gastrointestinal discomfort when ingested in large quantities. Even if gastrointestinal discomfort is noted when consumed in large quantities of D-allulose, it is not considered to be of toxicological significance since this type of symptom is usually transient

and is often associated with ingestion of non-digestible carbohydrates including dietary fiber (IOM, 2002).

A clinical study showed that the maximum tolerable levels in humans were 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females, with the mean value of 0.55 g/kg bw/day. These dosages correspond to 33.3 g/day for a 67 kg Asian male and 31.0 g/day for a 52 kg Asian female (Iida et al., 2007). These dosages also correspond to 45 - 46 g/person/day for an average American adult aged 20 years or older.

Table 10. Human Clinical Studies Referenced in Previous GRNs

Dosage	Length	Results	Reference
Up to 0.9 g/kg bw/d	6 days	No gastrointestinal symptoms up to 0.5 - 0.6 g/kg bw/d	Iida et al., 2007
15 g/d (5 g in tea, three times a day)	12 weeks	Positive impact on glycemic responses; no adverse effects were noted.	Hayashi et al., 2010
7.5 g in beverage	Single dose	Positive impact on glycemic and insulinemic responses; no adverse effects were noted.	Iida et al., 2008
Up to 340 mg/kg bw in beverage	Single dose	Metabolism study; no adverse effects were noted.	Iida et al., 2010

bw= body weight; d=day

6.D. SUMMARY

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

D-allulose has been safely used as a food ingredient around the world for a decade. As a result, a number of comprehensive reviews of the safety of D-allulose have been published (Chung et al., 2012b). In addition, the FDA has had no question on two GRAS notices related to the safety of D-allulose (GRN 400, FDA 2012; GRN 498, FDA, 2014).

6.D.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. Samyang Corp.'s D-allulose is manufactured under cGMP using common food industry materials and processes. Samyang Corp. uses a 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 concerning the chemistry of D-allulose. This GRAS determination is based on the data and information generally available and consented opinion about the safety of D-allulose. The literature indicates that D-allulose offers consumers benefits without adverse effects.

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 and meets all applicable purity standards.
2. Samyang Corp.'s D-allulose will be used as a sugar substitute and/or as a flavor modifier in food applications at use levels ranging from 2 to 100% in: selected bakery products (rolls, cakes, pastries, cakes, low calorie or dietetics), beverages (non-alcoholic, low or reduced calorie, sugar-free); cereals; chewing gums; confections and frostings; frozen dairy desserts (ice cream, soft serve, sorbet; low calorie, reduced calorie, sugar-free); yogurt and frozen yogurt (low calorie, reduced calorie, sugar-free); dressings for salads; gelatins, pudding and fillings (low calorie, reduced calorie, sugar-free); hard and soft candies (low calorie, reduced calorie, sugar-free); jams and jellies; sugar; sugar substitutes; sweet sauces and syrups (low calorie, reduced calorie, sugar-free) and fat based cream.
3. The LD_{50} value of D-allulose in rats is 15.8-16.3 g/kg. A chronic toxicity study 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 3% of the diet, the highest level tested.
4. A human clinical study showed that the maximum tolerable levels in humans were 0.5 g/kg bw/day for males and 0.6 g/kg bw/day for females. The only side effect of non-digestible carbohydrates, including D-allulose, is gastrointestinal discomfort when ingested in large quantities. This type of symptom is usually transient and is not considered to be of toxicological significance (IOM, 2002).
5. The proposed food use results in exposure at levels below those associated with any adverse effects. The EDI assessments are based on the assumption that Samyang Corp.'s D-allulose will replace currently marketed D-allulose. Thus, cumulative exposures are not expected. In addition, the EDIs presented in this notice are highly amplified estimates.

6. In the previous GRAS notices (GRN 400 and 498) to the FDA, the safety of D-allulose has been established in animal toxicity studies and mutagenicity studies, and is further supported by human clinical studies.
7. Additional animal studies published subsequent to the FDA GRAS notices continue to support the safety of D-allulose as a food ingredient.

Overall, there are no indications of significant adverse effects related to D-allulose in the publicly available literature. 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.E. 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-allulose preparations in foods and beverages, meeting appropriate specifications and used according to cGMP, is GRAS.

PART 7. DATA AND INFORMATION ARE GENERALLY AVAILABLE

7.1. DATA AND INFORMATION ARE GENERALLY AVAILABLE

All the references including animal and human studies are generally available.

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

Chung YM, Hyun Lee J, Youl Kim D, Hwang SH, Hong YH, Kim SB, Jin Lee S, Hye Park C. Dietary D-allulose reduced visceral fat mass in high-fat diet-induced obese rats. J Food Sci. 2012a;77:H53-H58.

Chung MY, Oh DK, Lee KW. Hypoglycemic health benefits of D-psicose. J Agric Food Chem. 2012b;60:863-9.

FDA. 2012. GRN 400. D-psicose, filed by CJ CheilJedang.

FDA. 2014. GRN 498, D-allulose (synonymous with D-psicose), filed by Matsutani Chemical Industry.

Han Y, Han HJ, Kim AH, Choi JY, Cho SJ, Park YB, Jung UJ, Choi MS. d-Allulose supplementation normalized the body weight and fat-pad mass in diet-induced obese mice via the regulation of lipid metabolism under isocaloric fed condition. Mol Nutr Food Res. 2016;60:1695-1706.

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-allulose in borderline diabetes and the safety of long-term ingestion by normal human subjects. Biosci Biotechnol Biochem. 2010;74:510-519.

Hossain MA, Kitagaki S, Nakano D, Nishiyama A, Funamoto Y, Matsunaga T, Tsukamoto I, Yamaguchi F, Kamitori K, Dong Y, Hirata Y, Murao K, Toyoda Y, Tokuda M. Rare sugar D-psicose improves insulin sensitivity and glucose tolerance in type 2 diabetes Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Biochem Biophys Res Commun. 2011;405:7-12.

Hossain A, Yamaguchi F, Matsunaga T, Hirata Y, Kamitori K, Dong Y, Sui L, Tsukamoto I, Ueno M, Tokuda M. Rare sugar D-allulose protects pancreas β-islets and thus improves insulin resistance in OLETF rats. Biochem Biophys Res Commun. 2012;425:717-723.

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-allulose prevents progression and development of diabetes in T2DM model Otsuka Long-Evans Tokushima Fatty rats. Drug Design Devel Ther. 2015;9:525-535.

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 Advanced Food Ingred. 2007;10: 15-19.

Iida T, Kishimoto Y, Yoshikawa Y, Hayashi N, Okuma K, Tohi M, Yagi K, Matsuo T, Izumori K. Acute D-allulose administration decreases the glycemic responses to an oral maltodextrin tolerance test in normal adults. J Nutr Sci Vitaminol. 2008;54:511-514.

Iida T, Hayashi N, Yamada T, Yoshikawa Y, Miyazato S, Kishimoto Y, Okuma K, Tokuda M, Izumori K. Failure of D-allulose absorbed in the small intestine to metabolize into energy and its low large intestinal fermentability in humans. Metabolism. 2010;59:206-214.

Institute of Medicine (IOM). 2002. Dietary Reference Intakes for energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein, and amino acids. National Academy Press, Washington, DC.

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:H1619-1626.

Karabinos JV. D-allulose, sorbose and tagatose. Adv Carbohydr Chem. 1952;7:99-136.

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;10:233-237.

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;48:512-516.

Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Metabolic effects of D-allulose in rats: Studies on fecal and urinary excretion and cecal fermentation. Asia Pac J Clin Nutr. 2003;12:225-231.

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;53:453-460.

Matsuo T, Izumori K. D-allulose inhibits intestinal α-glucosidase and suppresses glycemic response after carbohydrate ingestion in rats. Tech Bull Fac Agr., Kagawa Univ. 2006;58:27–32.

Matsuo T, Izumori K. D-Psicose inhibits intestinal alpha-glucosidase and suppresses the glycemic response after ingestion of carbohydrates in rats. J Clin Biochem Nutr. 2009;45:202-206.

Matsuo T, Ishii R, Shirai Y. The 90-day oral toxicity of D-allulose in male Wistar rats. J Clin Biochem Nutr. 2012;50:158-161.

Nagata Y, Kanasaki A, Tamaru S, Tanaka K. d-D-allulose, an epimer of d-fructose, favorably alters lipid metabolism in Sprague-Dawley rats. J Agric Food Chem. 2015;63:3168-3176.

Nishii N, Nomizo T, Takashima S, Matsubara T, Tokuda M, Kitagawa H. Single oral dose safety of D-allulose in dogs. J Vet Med Sci. 2016;78:1079-1083.

Nishii N, Nomizo T, Takashima S, Matsubara T, Tokuda M, Kitagawa H. Effects of D-allulose on glucose metabolism after the administration of sugar or food in healthy dogs. J Vet Med Sci. 2016;78:1657-1662.

Noda K, Oh T. Metabolism and disposition of erythritol after oral administration in rats. J Nutr. 1992; 122:1266-1272.

Ochiai M, Nakanishi Y, Yamada T, Iida T, Matsuo T. Inhibition by dietary D-allulose of body fat accumulation in adult rats fed a high-sucrose diet. Biosci Biotechnol Biochem. 2013;77:1123-1126.

Ochiai M, Onishi K, Yamada T, Iida T, Matsuo T. D-allulose increases energy expenditure and decreases body fat accumulation in rats fed a high-sucrose diet. Int J Food Sci Nutr. 2014; 65:245-250.

Oshima H, Kimura I, Izumori K. D-allulose contents in various food products and its origin. Food Sci Technol Res. 2006;12:137-143.

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-allulose. Drug Design Devel Ther. 2014;8:1955-1964.

Whistler RL, Singh PP, Lake WC. D-allulose metabolism in the rat. Carbohydr Res. 1974; 34: 200-202.

Yagi K, Matsuo T. The study on long-term toxicity of D-allulose in rats. J Clin Biochem Nutr. 2009;45:271-277.

7.2. DATA AND INFORMATION ARE NOT GENERALLY AVAILABLE

Not applicable.

APPENDIX A. CERTIFICATE OF ANALYSIS

1. Product 1. D-allulose syrup

Composition	Lot 1 (2015.08.08)	Lot 2 (2015.08.09)	Lot 3 (2015.08.20)	Analytical Method
Brix	75 Brix (%)			Brixmeter
pH	3.0 ~ 7.0			pH meter
D-Allulose*	24.63%	25.16%	25.04%	HPLC
Moisture	< 25%			AOAC941.14
Fructose or other sugars*	75.37%	74.84%	74.96%	HPLC
Total plate count	Negative	Negative	Negative	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0095 ppm	0.0048 ppm	0.0063 ppm	AOAC 2015.01
As	0.0071 ppm	0.0014 ppm	0.0024 ppm	AOAC 2015.01
Cd	0.0020 ppm	0.0011 ppm	0.0027 ppm	AOAC 2015.01

*Dry weight basis.

2. Product 2. D-allulose syrup

Composition	Lot 1 (2015.09.15)	Lot 2 (2015.09.30)	Lot 3 (2015.10.20)	Analytical Method
Brix	75 Brix (%)			Brixmeter
pH	3.0 ~ 7.0			pH meter
D-Allulose*	53.37%	53.22%	54.95%	HPLC
Moisture	< 25%			AOAC941.14
Fructose or other sugars*	46.63%	46.78%	45.05%	HPLC
Total plate count	Negative	Negative	Negative	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0040 ppm	0.0033 ppm	0.0074 ppm	AOAC 2015.01

D-Allulose (D-psicose)

As	0.0015 ppm	0.0015 ppm	0.0024 ppm	AOAC 2015.01
Cd	0.0038 ppm	0.0016 ppm	0.0013 ppm	AOAC 2015.01

*Dry weight basis.

3. Product 3. D-allulose syrup

Composition	Lot 1 (2015.09.15)	Lot 2 (2015.8.28)	Lot 3 (2015.10.06)	Analytical Method
Brix	75 Brix (%)			Brixmeter
pH	3.0 ~ 7.0			pH meter
D-Allulose*	95.90%	95.25%	96.19%	HPLC
Moisture	< 25%			AOAC 941.14
Fructose or other sugars*	4.10%	4.75%	3.81%	HPLC
Total plate count	Negative	Negative	Negative	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0024 ppm	0.0021 ppm	0.0028 ppm	AOAC 2015.01
As	0.0011 ppm	0.0006 ppm	0.0018 ppm	AOAC 2015.01
Cd	0.0022 ppm	0.0012 ppm	0.0014 ppm	AOAC 2015.01

*Dry weight basis.

4. Product 4-Crystalline **D-a**llulose, ≥98%

Composition	Lot 1 (2015.09.15)	Lot 2 (2015.9.30)	Lot 3 (2015.10.20)	Analysis Method
Moisture	0.15%	0.16%	0.14%	AOAC 941.14
D-Allulose*	99.44%	99.03%	99.43%	HPLC
Fructose or other sugars*	0.41%	0.81%	0.43%	HPLC
Total plate count	2.0×10^2	2.7×10^2	2.0×10^2	AOAC 2002.07
Salmonella	Negative	Negative	Negative	AOAC 989.14
Staphylococcus	Negative	Negative	Negative	AOAC 987.09
Coliforms	Negative	Negative	Negative	AOAC 991.14
Ash	0.00%	0.00%	0.00%	AOAC 900.02
Pb	0.0065 ppm	0.0054 ppm	0.0017 ppm	AOAC 2015.01

As	0.0027 ppm	0.0059 ppm	0.0062 ppm	AOAC 2015.01
Cd	0.0014 ppm	0.0016 ppm	0.0011 ppm	AOAC 2015.01

*Dry weight basis.