# Application to amend the Australia New Zealand Food Standards Code to permit D-Allulose as a novel food

**Samyang Corporation**
**Korea**

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## GENERAL REQUIREMENTS

### 1 Applicant Details

*(addressing section 3.1.1.B of the FSANZ Application Handbook)*

| | |
|---|---|
| Applicant | [redacted] |
| Contact | |
| Address | |
| Phone | |
| Email | |
| Nature of applicant's business | |
| Details of consultants associated with the application | |
| Primary contact for application | |

### 2 Purpose of the application

*(addressing section 3.1.1.C of the FSANZ Application Handbook)*

Samyang Corporation (Samyang) has submitted this application to request amendment to Schedule 25 of the Australia New Zealand Food Standards Code (the Code) to permit the sale of D-allulose as a novel food in Australia and New Zealand. D-allulose is intended to be added to foods as a low-energy substitute for conventional sugar ingredients, particularly sucrose. Samyang's D-allulose is manufactured by enzymatic epimerisation of fructose; however, the enzyme used in the manufacture of D-allulose is not permitted in the Code. Therefore, this application also requests approval of D-allulose-3-epimerase (also known as D-psicose-3-epimerase) harboured in the organism, *Microbacterium foliorum* to be used as a processing aid (and added to Schedule 18 of the Code).

Unlike traditional sugar, such as sucrose, D-allulose does not contribute significant metabolisable energy after consumption. To ensure appropriate labelling of the energy value of foods containing added D-allulose, the application also requests the establishment of a new energy factor of 1.0 kilojoule per gram (kJ/g) for D-allulose in section S11—2(3) of the Code.

The application also requests amendment of the Code's requirements for nutrition content claims for sugar(s) for foods containing added D-allulose. D-allulose is intended to be added to food as a sugar replacement ingredient, thereby reducing energy and conventional sugar content of these foods. Foods containing added D-allulose in place of conventional sugars (such as sucrose) will contain significantly less conventional sugars and metabolisable energy than traditionally sweetened counterparts. Samyang considers that foods containing added D-allulose should be permitted to carry the nutrition content claims for sugar(s) listed in the table to section S4—3, assuming the content of conventional sugars complies with the conditions listed in column 3 of the table.

Samyang is requesting that FSANZ investigate a mechanism to ensure that D-allulose is not subject to the conditions listed is column 3 of the table to section S4—3 for sugar(s). Recognising there are multiple sections in the Code that relate to the definition of sugar(s) and the conditions for making nutrition content claims for sugar(s), this application requests FSANZ investigate the most appropriate amendment to the Code, rather than specifying which section(s) of the Code should be amended. Section 3.4 of the application includes more detail on the Code's requirements relevant to sugar nutrition content claims. Section G.3 of the application discusses the request in more detail.

For clarity, Samyang is not requesting an amendment to the conditions for the 'unsweetened' nutrition content claim. D-allulose is a sweetener and making an 'unsweetened' claim for foods containing added D-allulose is not appropriate.

## 3 Justification for the application

*(addressing section 3.1.1.D of the FSANZ Application Handbook)*

As noted above, the application requests each of the following:

i. approval for the sale of D-allulose as a novel food
ii. approval for the use of D-allulose-3-epimerase expressed in the microorganism *M. foliorum* (SYG27B-MF) as a processing aid
iii. the addition of an energy factor for D-allulose (1.0 kJ/g) in section S11—2(3)
iv. a mechanism to enable foods containing added D-allulose to carry nutrition content claims relating to sugars.

The justification for each of these requests is expanded upon below.

### 3.1 Novel food justification

The Code (sections 1.1.1—10(5)(b) and 1.1.1—10(6)(f)) prohibits the sale of a novel food or foods containing a novel food as an ingredient unless the novel food is expressly permitted. Express permissions for the sale of novel foods are contained in Standard 1.5.1 – Novel foods, which states that novel foods listed in the table to S25-2[1] may be sold as food or ingredients in food. Samyang Corp. considers that D-allulose meets the Code's definition of novel food (section 1.1.2—8). S25-2 does not currently list D-allulose as a permitted novel food, meaning an application is required to seek permission to sell D-allulose as a novel food.

[1] S25 refers to Schedule 25 – Permitted novel foods

*D-allulose meets the definition of novel food*

'Novel food' is defined in section 1.1.2—8 of the Code:

(1) In this Code:

***novel food*** means a *non-traditional food that requires an assessment of the public health and safety considerations having regard to:

(a) the potential for adverse effects in humans; or
(b) the composition or structure of the food; or
(c) the process by which the food has been prepared; or
(d) the source from which it is derived; or
(e) patterns and levels of consumption of the food; or
(f) any other relevant matters.

***non-traditional food*** means:

(a) a food that does not have a history of human consumption in Australia or New Zealand; or
(b) a substance derived from a food, where that substance does not have a history of human consumption in Australia or New Zealand other than as a *component of that food; or
(c) any other substance, where that substance, or the source from which it is derived, does not have a history of human consumption as a food in Australia or New Zealand.

Samyang Corp. considers enzymatically produced D-allulose meets the definition of non-traditional food and novel food. Although D-allulose is naturally present in a variety of foods at low levels, there is not a history of human consumption of enzymatically produced D-allulose at the levels intended to be added to foods in Australia and New Zealand. Levels of D-allulose naturally present in foods range from 1.5 mg/100g in canned peaches up to 130 mg/100g of Worcester sauce (Oshima 2006). Table D.2.-1 provides a more substantial list of D-allulose content in foods.

The intended levels of use of D-allulose in foods are at least an order of magnitude greater than the natural levels of D-allulose in foods. Table D.1-1 lists the intended use levels of D-allulose in foods in Australia and New Zealand. In this context, Samyang Corp. considers the intended use levels of enzymatically produced D-allulose meets the definition of non-traditional food. In addition, noting the intended use levels will result in significantly altered patterns and levels of consumption of D-allulose, an assessment of public health and safety considerations is required in accordance with element (e) of the definition of novel food. That is, enzymatically produced D-allulose, at the intended levels of use identified in this application, meets the definition of novel food in section 1.1.2—8 of the Code.

This is consistent with the FSANZ Advisory Committee on Novel Food's (ACNF's) opinions on a variety of novel sugars. Most of the sugars considered novel by the ACNF have subsequently been approved via the FSANZ application process (alpha-cyclodextrin (Application A438), D-tagatose (A472), isomaltooligosaccharide (A1123), isomaltulose (A578) and trehalose (A453).

### 3.2 Processing aid justification

D-allulose is produced by conversion of fructose by an epimerase enzyme (D-allulose-3-epimerase) expressed in the microorganism *M. foliorum* (SYG27B-MF). Enzymes used in the production of food are generally regulated in Australia and New Zealand as processing aids. Section 1.1.1—10(6) of the Code states that food for sale must not have as an ingredient or a component, a substance that was used as a processing aid unless expressly permitted. Although the enzyme is not present in the final food, it appears likely, based on previous similar considerations by FSANZ, that it will be considered a processing aid in the context of the Code. D-allulose-3-epimerase from *M. foliorum* is not currently permitted by the Code for use as a processing aid. Therefore, this application requests amendment to S18—4(5) of the Code to permit the enzyme.

### 3.3 Energy factor justification

Section S11—2 of the Code lists energy factors to be used when calculating energy content of certain components of foods for labelling purposes. Section S11—2(2) lists carbohydrate as having a generic energy factor of 17 kJ/g. Unavailable carbohydrates, such as dietary fibre, have a generic energy factor of 8 kJ/g. Other food components have individual energy factors listed in section S11—2(3) where the energy factors differ from the generic energy factors listed in Section S11—2(2). Samyang considers D-allulose's energy factor is less than the generic energy factor for unavailable carbohydrate listed in section S11—2(2), but there is no specific energy factor for D-allulose listed in section 11—2(3). Therefore, this application includes a request for a new energy factor for D-allulose to be included in section S11-2(3). Further discussion of an energy factor for D-allulose is included in section G below.

### 3.4 Sugars nutrition content claim justification

Section 1.2.7—12 sets out requirements for nutrition content claims about properties of food listed in section S4—3 of the Code. Section S4—3 sets conditions for these types of nutrition content claims relating to sugars (see summary in Table 3.4-1).

Table 3.4-1. Sugars content claims and conditions in section S4—3 of the Code

| Nature of content claim | Condition |
|---|---|
| Low (and % Free) | The food contains no more sugars than:<br>(a) 2.5 g/100 mL for liquid food; or<br>(b) 5 g/100 g for solid food |
| Reduced or Light/Lite | The food contains at least 25% less sugars than in the same amount of reference food |
| No added | (a) The food contains no added sugars*, honey, malt, or malt extracts; and<br>(b) the food contains no added concentrated fruit juice or deionised fruit juice, unless the food is any of the following… |
| Unsweetened | (a) The food meets the conditions for a nutrition content claim about no added sugar; and<br>(b) the food contains no intense sweeteners, sorbitol, mannitol, glycerol, xylitol, isomalt, maltitol syrup or lactitol |

Section 1.1.2—2 of the Code defines sugars for the purposes of food labelling. An excerpt of the section 1.1.2—2 definition of sugars is included below:

> ***sugars***:
>
> (a) in Standard 1.2.7, Standard 1.2.8 and Schedule 4 (except where it appears with an asterisk as 'sugars*')—means monosaccharides and disaccharides; and
>
> (b) otherwise—means any of the following products, derived from any source:
>
> (i) hexose monosaccharides and disaccharides, including dextrose, fructose, sucrose and lactose;

D-allulose is a hexose monosaccharide and will meet both parts (a) and (b) of the labelling definition of sugars. Therefore, under the current Code provisions, D-allulose will be subject to the conditions for nutrition content claims relating to sugars in the table to section S4—3 of the Code. However, Samyang considers that D-allulose should be treated differently to other sugars (such as sucrose) because of the minimal metabolisable energy provided by D-allulose compared to these other sugars. Samyang considers that where D-allulose replaces conventional sugars in a food product, and the conditions for conventional sugars content in the food(s) is satisfied (in the table to section S4—3), the D-allulose content should not be taken into account in determining whether a nutrition content claim about sugar(s) can be made. That is, Samyang considers D-allulose should be excluded from consideration as a sugar for the purposes of making sugars nutrition content claims in accordance with section S4—3. The ability to make these nutrition content claims for sugar(s) is a very important aspect of marketing D-allulose as a sugar replacement ingredient, both for ingredient manufacturers such as Samyang and for food manufacturers marketing products to consumers.

This application therefore requests that FSANZ investigate a mechanism in the Code to enable foods containing added D-allulose to make nutrition content claims relating to sugar(s) (assuming the food satisfies the existing conditions for other sugars). Samyang notes that this could be achieved by amending the labelling definition of sugar(s) to exclude D-allulose, by including specific exceptions for D-allulose in the conditions in the table to section S4—3 or via an alternative mechanism that FSANZ considers more appropriate. Further discussion of sugars nutrition content claims relating to D-allulose is included in section G.3.

For clarity, Samyang is not requesting that foods containing added D-allulose be permitted to make 'unsweetened' nutrition content claims. D-allulose will be added to foods to provide sweetness.

## 3.5 Regulatory impact information

### *3.5.1 Costs and benefits of the application*

*a) Consumers*

D-allulose is intended to be added as a sugar substitute to reduced energy and reduced sugar foods. Replacing sugar with D-allulose will benefit consumers who seek foods and beverages with reduced energy from added sugars. Foods containing D-allulose as an ingredient may be priced at a premium over foods containing sugar and other traditional sugar-like ingredients. Cost differentials are common for newly developed ingredients. Consumers can choose to pay a premium for foods containing new ingredients or continue to purchase existing food formulations. Inclusion of a new energy factor in the Code for D-allulose will ensure food labels reflect the reduced energy content of D-allulose compared to other sweeteners, such as

sucrose. Consumers seeking lower energy products can target foods containing D-allulose, using nutrition information panel and other labelling or advertising information about D-allulose's energy content. If an energy factor for D-allulose is not included in the Code, the low-energy characteristics of D-allulose cannot be expressed on food labels.

*b) Industry*

As a low-energy carbohydrate, D-allulose will provide industry with an alternative to sucrose and other higher energy sugar ingredients. The contribution of sugar to the energy content of consumer diets is currently an area of key public and political interest in Australia and New Zealand. Sugar substitute ingredients present an opportunity for industry to meet consumer and public health demands for lower energy foods, particularly energy provided from sugars.

If Samyang Corp. obtains exclusive permission for its brand of D-allulose, industry competitors will not be permitted to sell different brands of D-allulose as a food or ingredient in food until Samyang Corp.'s 15-month period of exclusive permission expires; at which time the novel food permission for the sale of D-allulose becomes generic and freely available for competitors to take advantage of. However, industry competitors may also seek exclusive permission for other brands of D-allulose while FSANZ is assessing Samyang Corp.'s D-allulose application. Exclusive permission provides an exclusive capturable commercial benefit to an applicant; meaning Samyang Corp. must pay fees to FSANZ for the assessment of the application. Therefore, while Samyang Corp. will obtain benefit from an exclusive permission, the benefit is costing Samyang Corp. a significant amount of money in fees. Industry competitors will subsequently benefit from the generic novel food permission at the expiration of the exclusive permission period.

Inclusion of an energy factor for D-allulose in the Code will enable industry to reflect the reduced energy content of D-allulose on food labels. This will assist industry in communicating the lower energy content of D-allulose on food labels, particularly when comparing D-allulose to traditional sweetening ingredients, such as sucrose. If an energy factor for D-allulose is not included in the Code, food labels will be required to use higher energy values (for carbohydrate or unavailable carbohydrate) to express the energy content of D-allulose. This will not be an accurate reflection of the energy content of D-allulose and will make the use of D-allulose less attractive to food manufacturers, limiting the viability for D-allulose ingredient manufacturers marketing D-allulose in the Australian and New Zealand food market.

Industry will benefit from amendments to sugars nutrition content claim requirements that will enable D-allulose containing foods to carry claims such as 'low' or 'reduced' sugar and 'no added' sugars. This will make the use of D-allulose as a low energy sugar replacement ingredient more attractive for food manufacturers who can communicate the benefits of D-allulose's low energy properties to consumers. Greater industry uptake of D-allulose will also benefit D-allulose ingredient manufacturers.

*c) Government*

There should be limited cost impact on government agencies if D-allulose is permitted to be sold as a novel food. Samyang Corp. is paying fees for the FSANZ assessment of the application. Dietary guidelines in Australia and New Zealand recommend limiting the intake of added sugars. The use of low-energy carbohydrates, like D-allulose, as substitutes for sugars (such as sucrose) can help consumers align with these dietary guidelines. Inclusion of an energy factor in the Code for D-allulose will assist industry in communicating to consumers the lower energy

content of D-allulose compared to sugars such as sucrose; and enable consumers to choose foods containing D-allulose as an alternative low-energy carbohydrate.

### *3.5.2 Impact on international trade*

D-allulose can be added to foods in the United States, Korea and Japan. Samyang Corp. and other companies have obtained 'no questions' responses from the United States Food and Drug Administration in response to the conclusions of generally recognised as safe (GRAS) notifications. This application is part of a coordinated process of Samyang Corp. seeking approval for the sale of D-allulose in international jurisdictions, including the European Union and Canada (novel food applications). Permission in the Code to sell D-allulose as a novel food and novel food ingredient will facilitate international trade among jurisdictions in which D-allulose is already or soon to be permitted.

Inclusion of an energy factor in the Code for D-allulose will assist in ensuring that labelling of the energy content of D-allulose in Australia and New Zealand will reflect low-energy labelling of D-allulose foods in other countries, such as Korea, Japan (0 kJ/g) and the US (1.67 kJ/g). The lack of a distinct energy factor in the Code for D-allulose will make the marketing of D-allulose in Australia and New Zealand less attractive when compared to countries in which D-allulose can be labelled as a low or zero energy carbohydrate.

## 4 Information to support the application

The application contains supporting information in accordance with the Application Handbook's requirements, including:

- Chapter 3.1 General requirements for application
- Guideline 3.5.2 Novel foods
- Guideline 3.3.2 Processing aids
- Guideline 3.2.1 General food labelling
- Guideline 3.2.5 Nutrition information labelling
- Guideline 3.2.6.A.1 Nutrition content and health claims

The sections of this application that address the above guidelines of the Application Handbook are referenced below in Table 4-1.

Table 4-1 Cross references of Application Handbook requirements against the Application

| Handbook Guideline | Guideline details | Application section |
|---|---|---|
| 3.1 – General requirements | | 1-10 |
| 3.2.5 – Nutrition information labelling | 3.2.5.A – Information to support a change to the nutrition information labelling of a food | G.2 |
| | 3.2.5.B – Information to establish an energy factor | G.1 |
| 3.2.6 – Nutrition content and health claims | 3.2.6.A.1 – Information related to nutrition content claims in the table to section S4—3 | G.3 |
| 3.3.2 – Processing aids | 3.3.3.2.A – Technical information on the processing aid | B.8 |
| | 3.3.2.C – Information related to the safety of an enzyme processing aid | C.2 |

| Handbook Guideline | Guideline details | Application section |
|---|---|---|
| | 3.3.2.D – Additional information related to the safety of an enzyme processing aid derived from a microorganism | C.2 |
| | 3.3.2.F – Information related to the dietary exposure of the processing aid | D.6 |
| 3.5.2 – Novel foods | 3.5.2.A – Exclusive use of novel foods | A |
| | 3.5.2.B.1 – Type of novel food | B.1 |
| | 3.5.2.B.2 – Purpose of addition of the novel food | B.2 |
| | 3.5.2.B.3 – Physical and chemical properties of the novel food | B.3 |
| | 3.5.2.B.4 – Impurity profile of the novel food | B.4 |
| | 3.5.2.B.5 – Manufacturing process for the novel food | B.5 |
| | 3.5.2.B.6 – Specification for the novel food | B.6 |
| | 3.5.2.B.7 – Analytical method for detection of the novel food | B.7 |
| | 3.5.2.C – Safety of the novel food | C |
| | 3.5.2.C.4 – Single chemical entities (safety) | C.1 |
| | 3.5.2.C.6.1 – Safety of the source organism (foods derived from new source) | C.2 |
| | 3.5.2.D – Dietary exposure to the novel food | D |
| | 3.5.2.E – Nutritional and health impact of the novel food | E |
| | 3.5.2.F – Potential impact on consumer understanding and behaviour | F |

A literature search was undertaken for the preparation of this application, including information relating to safety. The search strategy was conducted on PubMed and was broad in nature, using the following terms: D-allulose OR D-psicose OR allulose OR psicose OR rare sugar. Approximately 3000 records were identified, with each abstract being investigated for relevance. D-allulose is intended to be added to foods as a low-energy substitute for sugar and the overarching purpose of the application is to seek permission to sell D-allulose as a novel food on this basis. This application does not include discussion of beneficial physiological or health related outcomes beyond the intended use of D-allulose as a low-energy sugar substitute ingredient. As an ingredient manufacturer, Samyang is focussed on marketing the sugar substitute properties of D-allulose to food manufacturers. Some studies cited in the application refer to effects of D-allulose on other parameters, such as blood sugar. These studies are provided to assist in the consideration of the safety of D-allulose and to support other aspects, such as the calculation of the energy factor. However, Samyang considers additional research is likely required to support a purpose of addition of D-allulose to foods beyond its use as a low-energy sugar substitute ingredient.

## 5 Assessment procedure

*(addressing section 3.1.1.F of the FSANZ Application Handbook)*

Samyang considers the application should be assessed under the general procedure.

## 6 Confidential commercial information (CCI)

*(addressing section 3.1.1.G of the FSANZ Application Handbook)*

The application contains annexes that Samyang considers contain confidential commercial information (CCI). This information is of commercial value to Samyang Corp. and has not been publicly released to date. Public release of this information can reasonably be expected to diminish the commercial value of this information to Samyang Corp. These annexes have been provided to FSANZ to assist in its assessment and summary information is included in the application where possible. The following annexes are considered by Samyang to be CCI:

- Annex E – relating to testing to confirm no source organism is detected in commercial D-allulose products
- Annex F – relating to certain safety tests conducted on the source organism
- Annex H – relating to study report on teratogenicity
- Annex I – relating to a study on serum
- Annex J – relating to the cultivation and immobilisation of the production organism
- Annex K – relating to a study on biogenic amine production
- Annex L – relating to full study report of 90-day oral toxicity test
- Annex M – relating to full study report of reproductive toxicity test
- Annex N – relating to study report of reverse mutation test
- Annex O – relating to study report of chromosome aberration test
- Annex P – relating to study report of in vivo micronucleus test

## 7 Other confidential information

*(addressing section 3.1.1.H of the FSANZ Application Handbook)*

No other confidential information is included in this application.

## 8 Exclusive capturable commercial benefit (ECCB)

*(addressing section 3.1.1.I of the FSANZ Application Handbook)*

Samyang is requesting exclusive permission for its Allulosa brand of D-allulose in various classes of food (section A contains additional information on the request for exclusive permission). Granting of an exclusive permission by FSANZ will confer an ECCB on the applicant. No other aspects of this application would confer an ECCB on the applicant.

## 9 International and other standards

*(addressing section 3.1.1.J of the FSANZ Application Handbook)*

### 9.1 International standards

There are no international standards for novel foods or for the use of D-allulose as a food and food ingredient. However, individual jurisdictions have relevant requirements which are outlined below in section 9.2.2. Similarly, there are no international standards for the calculation of energy factors for food components such as D-allulose, which is not metabolised like other simple carbohydrates. The Codex Alimentarius Commission's Guidelines on Nutrition Labelling

(CAC/GL 2-1985) includes guidance on calculation of energy for carbohydrates (17kJ/g), protein (17 kJ/g), Fat (37 kJ/g) and alcohol (29 kJ/g), but not for other nutrients that are metabolised differently (Codex 2017[2]).

## 9.2 Other national standards or regulations

### 9.2.1 Australia and New Zealand

There are no relevant standards in Australia and New Zealand, other than the Code requirements for novel foods identified above.

### 9.2.2 International

D-allulose products are currently sold in the United States (US), Japan, and Korea.

#### *9.2.2.1 United States*

In the US, several sources of D-allulose have established a Generally Recognised as Safe (GRAS) status. The US Food and Drug Administration (FDA) has issued 'no questions' letters for four GRAS notifications related to food uses of D-allulose (GRN 400; GRN 498; GRN 693 and GRN 828). In these GRAS notifications, 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 0.5-0.6 g/kg bw/day is safe. Samyang Corp.'s D-allulose from *M. foliorum* is the subject of GRN 828 which received FDA's no question letter. *M. foliorum* (SYG27B-MF) harbouring D-allulose-3-epimerase enzyme activity does not require separate approval in the US.
Table 9.2.2.1-1 summarises previous GRAS notices and the current notice for D-allulose.

Table 9.2.2.1-1. Summary of FDA GRAS Notifications

| GRN | Company | Production microorganism harbouring enzyme* | Intended use | EDI, 90th pctl for all users |
|---|---|---|---|---|
| 828 | Samyang Corp. | Non-GMO *Microbacterium foliorum* | 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 |
| 693 | Samyang Corp. | GMO *Corynebacterium glutamicum* | | |
| 400 | CJ Cheiljedang | GMO *Corynebacterium glutamicum* | As a sugar substitute in foods at use levels ranging from 2 to 10%. | 28.5 g/person/day or 0.36 g/kg bw/day |
| 498 | Matsutani | GMO *Streptomyces violaceoruber* | 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 |

*Enzyme= D-allulose 3-epimerase; bw= body weight; GRAS= generally recognised as safe; EDI = estimated dietary intake; pctl=percentile.

[2] Adopted in 1985 and last amended in 2017

Copies of each of these original GRAS notifications have been provided with this application and are available online:
GRN 400: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=400.
GRN 498: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=498.
GRN 693: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=693.
GRN 828: https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=828.

The energy value of D-allulose in the US has not been formally established for labelling purposes. However, the FDA has released guidance for industry in which the agency notes it will exercise enforcement discretion for the use of a general factor of 0.4 calories per gram (approximately 1.7 kJ per gram) for allulose when determining 'calories' on food label (FDA 2020). The FDA's guidance also advises that a discretionary approach will be taken to the exclusion of D-allulose from the amount of 'Total Sugars' and 'Added Sugars', pending future rulemaking amendments regarding both of these aspects of sugar labelling.

#### *9.2.2.2 European Union*

Samyang has submitted a novel food application to the European Union (EU) for assessment. The EU is currently assessing Samyang's and three other applications for the approval of allulose as a novel food.

#### *9.2.2.3 Canada*

Samyang Corp. has submitted a novel food premarket notification to Health Canada for D-allulose (2020).

#### *9.2.2.4 Other*

D-allulose is permitted to be marketed in South Korea. M. foliorum harbouring D-allulose-3-epimerase enzyme activity has been approved in Korea. D-allulose is considered to be a zero-energy carbohydrate in South Korea. That is, the energy value to be used for labelling of foods containing D-allulose in South Korea is zero (0) kcal/g; as set out in the Ministry of Food And Drug Safety's 'Foods Labelling Standards' (MFDS 2016 – p157).

D-allulose has been marketed in Japan without the need for regulatory approval. D-allulose's energy factor for food labelling purposes in Japan is also 0 kcal/g.

D-allulose was approved in Mexico (2017) as a non-caloric sweetener by the Federal Commission for the Protection against Sanitary Risk:
https://www.gob.mx/cms/uploads/attachment/file/628596/ANEXO_VII.pdf.

## 10 Statutory Declaration

A signed statutory declaration accompanies this application (Annex R).

## NOVEL FOOD REQUIREMENTS

### A Exclusive use of novel foods

*(addressing section 3.5.2.A of the FSANZ Application Handbook)*

The application is seeking exclusive permission for Samyang Corporation's 'Allulosa' brand D-allulose in the classes of food identified in Table A.1. These classes of food are broader than the specific intended uses described in section D.1 of the application, which includes a break-down of the classes of food to assist with dietary modelling that FSANZ is likely to undertake. Samyang does not seek permission to add D-allulose to foods such as infant formula, formulated supplementary foods for young children, formulated meal replacement products or to raw commodity products such as meat, fruit, vegetables and milk.

Samyang is requesting a 15-months period of exclusive permission.

Table A.1. Classes of food for exclusive permission for 'Allulosa' brand D-allulose

| Food class |
|---|
| Bakery products |
| Beverages (water based, non-alcoholic) |
| Breakfast cereals and cereal based bars |
| Chewing gum |
| Icings and frostings |
| Frozen dairy desserts |
| Yogurt |
| Dressings for salads |
| Gelatins, pudding and fillings |
| Hard and soft candies/confectionery |
| Jams and jellies |
| Sugar products |
| Sugar substitutes |
| Sweet sauces and syrups |
| Fat-based cream |

### B Technical information on the novel food

*(addressing section 3.5.2.B of the FSANZ Application Handbook)*

#### B.1 Information on the type of novel food

*(addressing section 3.5.2.B.1 of the FSANZ Application Handbook)*

D-allulose is a monosaccharide sugar that is an epimer of D-fructose. D-allulose is naturally present in small amounts in a variety of foods. It can also be synthesised by enzymatic epimerisation of D-fructose. D-allulose has a lower energy content than traditional sugars such as sucrose. As such, D-allulose is intended to be added as an alternative to sugar and other higher energy carbohydrates in order to reduce the energy content of foods. D-allulose is also intended to be sold as a sugar replacement product to consumers, in both powder and syrup form (see section D.1 for detailed description of intended uses). The FSANZ Application Handbook identifies sugars as 'dietary macro-components'. Therefore, the information on the safety of D-allulose addresses the dietary macro-component requirements in guideline 3.5.2.C.4 of the Application Handbook (section C below).

## B.2 Information on the purpose of adding a novel food ingredient to food

*(addressing section 3.5.2.B.2 of the FSANZ Application Handbook)*

The purpose of adding D-allulose to food is as a low-energy alternative to conventional carbohydrate ingredients, such as sucrose. This will enable foods containing D-allulose to maintain beneficial flavour and other properties while containing significantly less energy than counterpart foods containing sucrose and other higher energy carbohydrates. The combination of lower energy content and technical functionality of D-allulose makes it an attractive alternative food ingredient to traditional sugar ingredients such as sucrose. Evidence of D-allulose's lower available energy content is included in section G. Despite its lower energy content, D-allulose fulfils technical properties in foods like traditional sugar ingredients, including browning, providing bulk and sensory properties, and facilitating foaming and gelling properties of egg white proteins in products such as nougats, cakes and custards. These properties are discussed in section B.3.2.

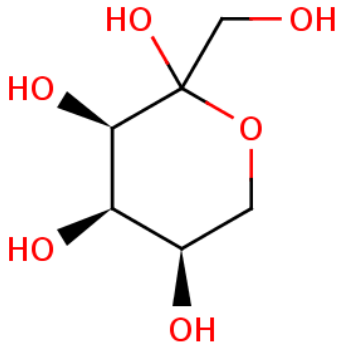
D-allulose is intended to replace sugar as an ingredient in reduced energy foods. D-allulose is not metabolised like other simple sugars in animals and humans, meaning that significantly less energy is available from the consumption of D-allulose compared to sugars, such as sucrose, that are commonly added to foods. D-allulose is 70% as sweet as sucrose. Therefore, total replacement of sugars (such as sucrose) with D-allulose is likely to require additional sweeteners, including intense sweeteners, to be added in combination with D-allulose in order to provide a similar sweetness profile to sucrose. Total or partial replacement of sugars with D-allulose can maintain desired sweetness and technical profiles of sweetened foods while also reducing energy content.

## B.3 Information on the physical and chemical properties of the novel food or novel food ingredient

*(addressing section 3.5.2.B.3 of the FSANZ Application Handbook)*

D-allulose is a monosaccharide epimer of D-fructose, isomerised at C-3. The physical and chemical properties of D-allulose are included in Table B.3-1. The stability of D-allulose is discussed in section B.3.1. Other technical aspects relating to the use of D-allulose as an ingredient in foods, including its browning properties, bulk and sensory properties, and facilitating foaming and gelling properties of egg white proteins in products such as nougats, cakes and custards is discussed in section B.3.2.

Table B.3-1. Physical and chemical properties of D-allulose

| | |
|---|---|
| Common name | D-allulose, D-psicose, pseudo-fructose |
| Chemical name | D-ribo-2-ketohexose |
| CAS registry number | 551-68-8 |
| Empirical formula | $C_6H_{12}O_6$ |
| Structural formula |   |
| Molecular weight | 180.156 |
| Melting point (°C) | 114-115 |
| Specific optical rotation | +4.7 (Crystal) |
| Solubility in water (25°C) | 324.2 g/100 mL |
| Specific gravity (g/cm$^3$, 74% solution) | 1.35 |
| Particle size | 50-500 micrometres. The functionality of D-allulose is not related to particle size |

*B.3.1 Stability*

Stability testing of D-allulose syrups containing no less than 8% (syrup L) and no less than 62% (syrup H) D-allulose, respectively, as well as crystalline powder containing no less than 98% D-allulose, were conducted under different storage conditions. Five batches of each preparation were stored at 25°C, 35°C, and 45°C with D-allulose content being measured at various intervals during a period ranging from 15 months for the D-allulose syrup H, up to 28 months for the D-allulose syrup L and 29 months for the 98% crystalline D-allulose powder. Data based on averages of the five batches for each preparation is presented in Tables B.3.1-1 to B.3.1-3 below.

As shown in Table B.3.1-1, the D-allulose crystalline powder showed negligible loss of D-allulose over the period of testing. The D-allulose crystalline powder (Product 1) experienced little change in D-allulose content over 29 months of storage at each of the three temperatures. At 25, 35, and 45°C, 99.9-100% recovery was reported at month 29 when compared to the baseline value. It is reasonable to expect that the powder form of D-allulose is stable for 2-3 years.

The D-allulose syrup H (Product 2) contained an average of 73.5% D-allulose on an as-is basis at the beginning of testing. After 15 months stored at 25°C, 96.8% recovery was reported when compared to the baseline value. Greater loss of D-allulose was experienced at 45°C with D-allulose recovery of 84.1% after 15 months compared to the baseline value.

The D-allulose syrup L (Product 3) contained an average of 12.05% D-allulose based on an as-is basis at the beginning of testing. After 28 months stored at 25°C, approximately 95.7%

recovery was reported when compared to the baseline value. After 28 months storage at 45°C, recovery was reduced to about 73.8% when compared to the baseline value. Syrups will be supplied in excess of 115% of the claim value at the time of shipment.

Overall, it is reasonable to conclude that crystalline D-allulose powder and syrups are stable for 24 and 15 months, respectively, at ambient temperature.

Table B.3.1-1. Summary of storage results of D-allulose crystalline powder (Allulose ≥98%) at various temperatures*

| Storage Duration (month) | Allulose (%, w/w) | | | % Recovery | | |
|---|---|---|---|---|---|---|
| | 25°C | 35°C | 45°C | 25°C | 35°C | 45°C |
| 0 | 99.76 | 99.76 | 99.76 | 100.0 | 100.0 | 100.0 |
| 1-2.3 | 99.76 | 99.73 | 99.72 | 100.0 | 100.0 | 100.0 |
| 3.3-4.3 | 99.74 | 99.71 | 99.70 | 100.0 | 100.0 | 99.9 |
| 6-8 | 99.74 | 99.72 | 99.69 | 100.0 | 100.0 | 99.9 |
| 10-12 | 99.74 | 99.70 | 99.69 | 100.0 | 99.9 | 99.9 |
| 13-16.5 | 99.74 | 99.69 | 99.67 | 100.0 | 99.9 | 99.9 |
| 18-20.5 | 99.71 | 99.68 | 99.66 | 100.0 | 99.9 | 99.9 |
| 22.5-24 | 99.75 | 99.70 | 99.70 | 100.0 | 99.9 | 99.9 |
| 26-29 | 99.76 | 99.74 | 99.73 | 100.0 | 100.0 | 100.0 |

*Average of 5 lots; on a dry weight basis.

Table B.3.1-2. Summary of storage results of D-allulose syrup H (Product 2) on an as-is basis

| Storage Duration (month) | Allulose (%, w/w) | | | % Recovery | | |
|---|---|---|---|---|---|---|
| | 25°C | 35°C | 45°C | 25°C | 35°C | 45°C |
| 0 | 73.5 | 73.5 | 73.5 | 100.0 | 100.0 | 100.0 |
| 0.5-1 | 73.1 | 70.2 | 67.9 | 99.5 | 95.6 | 92.5 |
| 1.5-2 | 72.8 | 68.0 | 66.6 | 99.1 | 92.6 | 90.6 |
| 2.5-3 | 72.5 | 67.2 | 65.9 | 98.7 | 91.5 | 89.8 |
| 3.5-4 | 72.1 | 66.6 | 65.5 | 98.2 | 90.6 | 89.2 |
| 6-7 | 72.4 | 66.4 | 65.4 | 98.6 | 90.4 | 89.1 |
| 8-9 | 71.9 | 65.5 | 64.3 | 97.8 | 89.2 | 87.5 |
| 10-12 | 71.7 | 64.9 | 63.5 | 97.6 | 88.4 | 86.4 |
| 15 | 71.1 | 64.1 | 61.8 | 96.8 | 87.3 | 84.1 |

*Average of 5 lots; as-is basis. The above values were multiplied by 0.751 (the average moisture content value was 24.9%).

Table B.3.1-3. Summary of storage results of D-allulose syrup L (Product 3) on an as-is basis

| Storage Duration (month) | Allulose* (%, w/w) | | | % recovery | | |
|---|---|---|---|---|---|---|
| | 25°C | 35°C | 45°C | 25°C | 35°C | 45°C |
| 0.0 | 12.05 | 12.05 | 12.05 | 100.0 | 100.0 | 100.0 |
| 0.5-1.0 | 12.03 | 12.01 | 11.96 | 99.9 | 99.7 | 99.3 |
| 2.0-2.5 | 11.98 | 11.92 | 11.53 | 99.5 | 99.0 | 95.7 |
| 4-5.5 | 12.01 | 11.86 | 11.38 | 99.7 | 98.4 | 94.5 |
| 6.0-7.5 | 11.98 | 11.75 | 11.12 | 99.5 | 97.6 | 92.3 |
| 9-10.5 | 11.90 | 11.63 | 10.77 | 98.8 | 96.6 | 89.4 |
| 12-13.5 | 11.84 | 11.46 | 10.53 | 98.3 | 95.2 | 87.4 |
| 15-16 | 11.78 | 11.26 | 10.09 | 97.8 | 93.4 | 83.8 |
| 18-19 | 11.72 | 11.11 | 9.77 | 97.3 | 92.2 | 81.1 |
| 20-22 | 11.67 | 10.99 | 9.50 | 96.9 | 91.2 | 78.9 |
| 24 | 11.56 | 10.74 | 8.96 | 96.0 | 89.1 | 74.4 |
| 28 | 11.53 | 10.64 | 8.90 | 95.7 | 88.4 | 73.8 |

*Average of 5 lots; on as-is basis. The above values were multiplied by 0.761 (average moisture value was 23.9%)

### *B.3.1.1 Stability in food matrices*

The stability of D-allulose under various conditions of use also has been evaluated. For example, cereal fortified with D-allulose was packaged in Tetrapaks™ and stored at ambient temperature conditions (25°C and 35°C) for 85 days. A sensory panel did not detect fresh fish odour or flavour over the duration of the trial, and the samples remained organoleptically acceptable. D-allulose content did not fall over the test period, regardless of storage temperature. The loss of moisture and water activity in the cereal product over the 85 days is likely to have increased the relative concentration of D-allulose identified in tables B.3.1.1-1 and B.3.1.1-2 (rather than the quantity of D-allulose increasing over time). Other monosaccharides, such as D-fructose and glucose, are stable in dried foods for over 24 months at ambient temperature. It is expected that D-allulose is as stable as other monosaccharides.

Table B.3.1.1-1. Results of Storage of Cereal at 25°C

| Storage duration (day) | Microbiological | Physicochemical | | | |
|---|---|---|---|---|---|
| | ***Bacillus cereus*** | Moisture (%) | Water activity | Allulose (mg/mL) | Allulose % recovery |
| 0 | ND | 5.82±0.01 | 0.45±0.00 | 47.58±3.59 | 100 |
| 85 | ND | 4.01±0.01 | 0.30±0.00 | 51.42±0.02 | 108.1 |

Table B.3.1.1-2. Results of Storage of Cereal at 35°C

| Storage duration (day) | Microbiological | Physicochemical | | | |
|---|---|---|---|---|---|
| | ***Bacillus cereus*** | Moisture (%) | Water activity | Allulose (mg/mL) | Allulose % recovery |
| 0 | ND | 5.82±0.01 | 0.45±0.00 | 47.58±3.59 | 100 |
| 85 | ND | 3.11±0.01 | 0.21±0.00 | 47.17±0.05 | 99.1 |

### B.3.2 Other technical properties of D-allulose in foods

D-allulose is 70% as sweet as sucrose, so substituting 1:1 D-allulose for sucrose will not provide the same sweetness profile as sucrose. Where maintaining a sucrose sweetness profile is important, food manufacturers may choose to use a combination of D-allulose and intense sweeteners, rather than using a greater quantity of D-allulose, in order to maximise the lower energy content of final foods. Food manufacturers may also choose to use a 1:1 mixture of D-allulose and sucrose, which has been reported to have the same sweetness profile as sucrose (Wee et al. 2018).

D-allulose has been shown to provide technical benefits when added to foods, particularly as a substitute for sucrose. Sun et al. (2008) demonstrated that D-allulose exhibited improved performance over sucrose in contributing to the foaming properties of egg white proteins in aerated foods (which is important in foods such as cakes, whipped cream, nougat and chocolate mousse). Sun et al. also highlighted additional benefits of using D-allulose compared to artificial intense sweeteners in aerated foods, such as offering greater bulking and sensory properties while also providing no energy. Sun et al. (2006) demonstrated that D-allulose, used as a substitute for sucrose, in a custard pudding dessert, exhibited higher antioxidant activity than traditional custard pudding desserts that use sucrose and fructose as the sugar ingredients; and exhibited outstanding gelling characteristics. In addition, Sun et al. (2004) demonstrated that D-allulose, when combined with ovalbumin, produced stronger cross-linking activity and faster browning than glucose and fructose controls, indicating that D-allulose can improve gelling qualities of ovalbumin (compared to glucose and fructose) in sweetened food products.

#### *B.3.3.3 Conclusion*

D-allulose is a monosaccharide that provides the same or improved functional properties of conventional sugar ingredients in foods but with lower energy content. Stability studies indicate that D-allulose crystalline powder and syrups are stable under typical storage conditions. In addition, D-allulose in a cereal food matrix was stable for 85 days at accelerated storage conditions and is expected to have similar stability in foods as other monosaccharide ingredients such as fructose and glucose. Compared to intense sweeteners, D- allulose provides greater bulking and sensory properties in foods. Compared to sucrose, glucose and fructose, D-allulose also provides improved properties when combined with egg white proteins (foaming, cross-linking and browning) and as a replacement for sucrose and fructose in custard pudding desserts (higher antioxidant activity and strong gelling activity).

## B.4 Information on the impurity profile for a typical preparation

*(addressing section 3.5.2.B.4 of the FSANZ Application Handbook)*

### *B.4.1 Raw materials and processing aids*

The manufacture of D-allulose is described in detail in section B.5 below. Table B.4.1-1 lists the raw materials and processing aids used in the manufacturing process in addition to the calcium alginate gel bead (immobilised cell system) containing *M. foliorum*.

Table B.4.1-1 Raw materials used in the manufacture of D-allulose

| Materials | CAS Number |
|---|---|
| Fructose syrup | 57-48-7 |
| Manganese sulphate | 7785-87-7 |
| Activated carbon | 7440-44-0 |
| Sodium hydroxide | 1310-73-2 |

Filtration and ion exchange processes are undertaken after the conversion of D-fructose to D-allulose to remove impurities (such as calcium, manganese, magnesium, chloride, sulphate, amino acids, peptides proteins). Product specifications and analytical results of non-consecutive batches of D-allulose demonstrate low levels of environmental impurities, including heavy metals and microorganisms. Section B.6 includes more detailed description of product specifications and analytical testing results for D-allulose products.

### *B.4.2 No detection of residual M. foliorum SYG27B-MF in D-allulose*

Samyang Corp. has commissioned testing to confirm that no residual *M. foliorum* is present in commercial D-allulose products. Annex E (CCI) provides detail on the tests conducted. In summary, Samyang Corp. provided five independent batches of each of the following D-allulose preparations for testing: D-allulose syrups containing no less than 8% and no less than 62% D-allulose respectively; and crystalline powder containing no less than 98% D-allulose. Viable cell counts on D-allulose mediums after six days incubation detected no colony forming units of *M. foliorum*. In addition, after applying a primer that specifically acts on *M. foliorum* SYG27B-MF, PCR and electrophoresis methods confirmed the absence of DNA from *M. foliorum* in the D-allulose ingredient samples.

## B.5 Manufacturing process for a novel food ingredient

*(addressing section 3.5.2.B.5 of the FSANZ Application Handbook)*

Section B.5.1 describes the manufacturing process of D-allulose. D-allulose is produced by conversion of fructose by an epimerase enzyme (D-allulose-3-ipimerase) expressed in the microorganism *M. foliorum* (SYG27B-MF). Enzymes used in the production of food are generally regulated in Australia and New Zealand as processing aids. Section 1.1.1—10(6) of the Code states that food for sale must not have as an ingredient or a component, a substance that was used as a processing aid. Although the enzyme is not present in the final food, it appears likely, based on previous similar considerations by FSANZ, that it will be considered a processing aid in the context of the Code. D-allulose-3-epimerase from *M. foliorum* is not currently permitted by the Code for use as a processing aid. Therefore, this application addresses requirements in the Application Handbook related to enzyme processing aids. Sections B.5.2 and B.5.3 include consideration of these requirements.

*B.5.1 Manufacturing process for D-allulose*

The steps involved in the production of D-allulose are described below and outlined in Figure B.5-1. Samyang Corp.'s D-allulose is manufactured from fructose in an aqueous solution by enzymatic epimerisation in the presence of manganese ions or magnesium ions.

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 neturalized fructose syrup is passed through an immobilized cell system (calcium alginate gel bead entrapped with non-GMO *M. foliorum* (SYG27B-MF) cell possessing D-allulose-3-epimerase activity. The fructose is then converted to D-allulose at 50℃ in the presence of manganese ions, which promote the enzymatic epimerization process. Samyang uses manganese sulfate as a source of manganese.
3. For decolorization and desalting, the D-allulose solution is optionally 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 cation 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 allulose syrup ≥8% (on an as-is 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 ≥68 ºBx to produce allulose syrup ≥62% (on an as-is basis).
7. The final concentrated product is pumped into a crystallizer.
8. The crystalline D-allulose is separated by basket centrifugation, washed by spraying distilled water, and finally dried in a dryer to give the final D-allulose concentration of ≥98%.

The purification process includes the application of the accepted food processing techniques of neutralization and bleaching/ deodorizing, as appropriate, to provide D-allulose that may be used for foods. The D-allulose plant was designed specifically for D-allulose and uses unit processes that are standard in the edible carbohydrate industry. The factory operates in accordance with standard GMPs. The premises and the manufacturing processes have qualified for International Standards Organisation (ISO) 9001:2000 and Hazard Analysis and Critical Control Point (HACCP) certification and are operated to comply with a strict environmental protection code. Several steps in the purification process are described in detail below. Certificate for HACCP is shown in Annex B. Samyang's ISO certificate is shown in Annex C.

In the decolorization step, the D-allulose ingredients are treated with activated carbon and clay, primarily to adsorb pigments, but this also is effective in reducing the content of oxidation (breakdown) products, trace metals, phosphorus and, to a lesser extent, sulphur compounds.

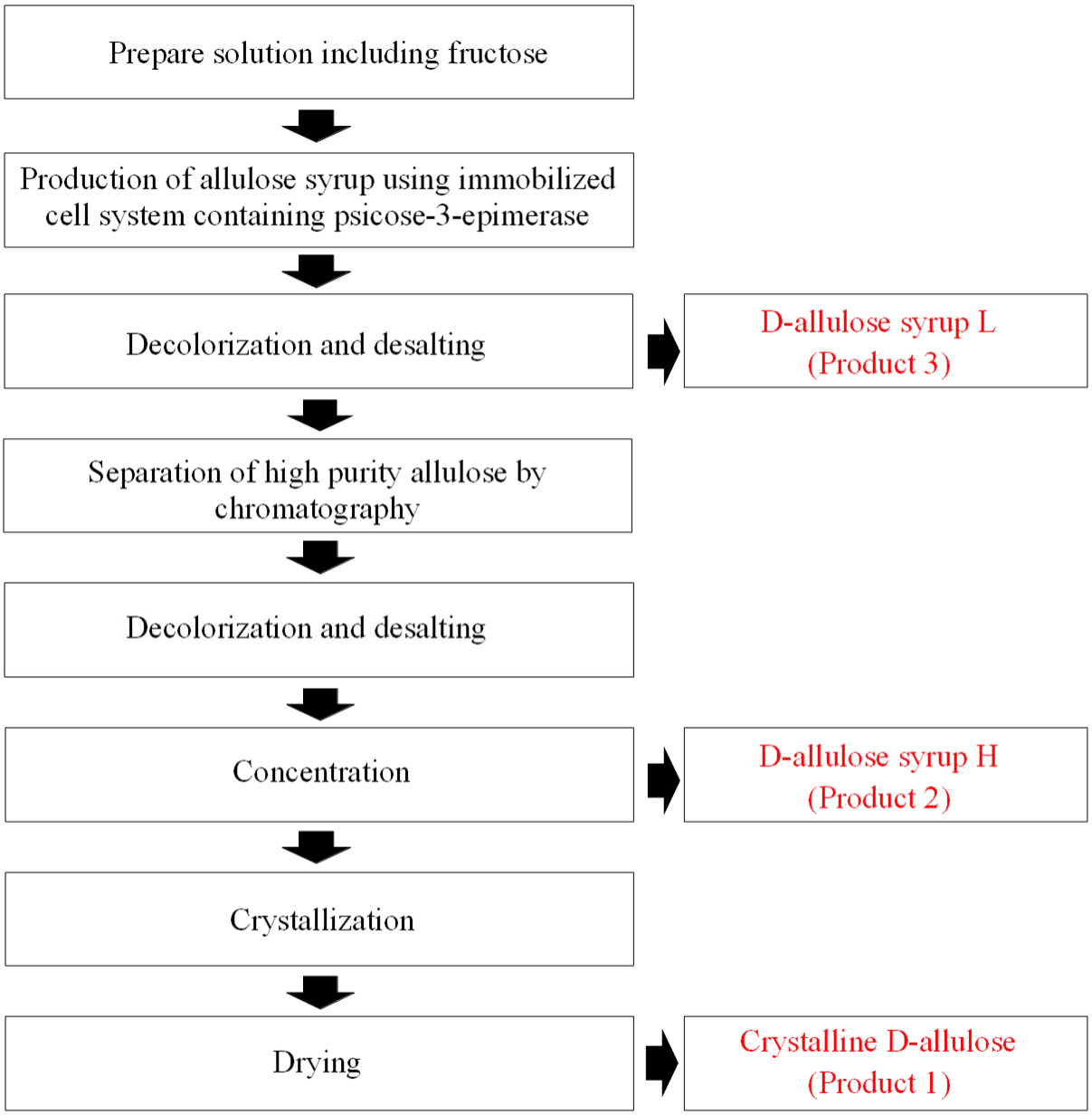



**Figure B.5-1 Processing Steps for the Preparation of D-Allulose**

## B.6 Specification for identity and purity for a novel food ingredient

*(addressing section 3.5.2.B.6 of the FSANZ Application Handbook)*

There are no specifications for D-allulose listed in the published sources identified in Schedule 3 – Identity and purity of the Code. Specifications for Samyang Corp.'s crystalline D-allulose and ≥62%(as-is) and ≥8%(as-is) D-allulose syrups are provided in Table B.6-1

Table B.6-1. Specifications and analytical values of D-allulose crystalline powder and syrups (Products 1 to 3)

| **Composition** | **Specification** | | | **Method of Analysis** |
|---|---|---|---|---|
| **Product name** | **Crystalline D-allulose (Product 1)** | **D-allulose Syrup H (Product 2)** | **D-alluose Syrup L (Product 3)** | - |
| Appearance | Powder | Clear yellow liquid | | Visual |
| Odor | No odor | No odor | No odor | - |
| D-allulose, g/100g, dry wt. basis | ≥98 | ≥90 | ≥10 | HPLC |
| D-allulose, g/100g, as-is basis | ≥98 | ≥62 | ≥8 | HPLC |
| Moisture, g/100g | ≤2 | ≤32 | ≤25 | AOAC 941.14 |
| Brix | NA | ≥68 | ≥75 | Brix refractometer |
| pH | NA | 3.0 – 7.0 | 3.0 – 7.0 | pH meter |
| Ash, g/100g | ≤0.1 | ≤0.1 | ≤0.1 | AOAC 900.02 |
| Pb, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | AOAC 2015.01 |
| As, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | AOAC 2015.01 |
| Cd, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | AOAC 2015.01 |
| Hg, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | AOAC 2015.01 |
| Total plate count, CFU/g | ≤1,000 | ≤1,000 | ≤1,000 | AOAC 990.12 |
| Coliforms, CFU/g | ND | ND | ND | AOAC 991.14 |
| Salmonella, CFU/25g | ND | ND | ND | AOAC 989.14 |
| *Staphylococcus aureus, CFU/g* | ND | ND | ND | AOAC 987.09 |

CFU = colony forming units, ND = not detected, NA = not available

### B.6.1 Product analyses

Summaries of analytical data for representative batches of D-allulose, manufactured as described in section B.5 and demonstrating compliance with the above specifications are provided in Tables B.6.1-1 to B.6.1-3. Table B.6.1-4 shows that other components in Product 3 (D-allulose Syrup L) are mostly fructose and glucose (mean, 42.9g/100g and 36.5g/100g respectively, on a dry weight basis). Table B.6.1-5 shows residual concentrations of magnesium and manganese in the finished D-allulose ingredients were less than 0.1 mg/kg; evidence that these minerals are effectively removed during the purification process. Certificates of analysis are presented in Annex A.

Table B.6.1-1. Specifications and Analytical Values of Product 1 (Crystalline D-allulose, ≥98%)

| Composition | Specification | Lot Number | | | | |
|---|---|---|---|---|---|---|
| | | 2018 0516 | 2018 0716 | 2018 1202 | 2019 0228 | 2019 0515 |
| Appearance | Crystalline powder | Crystalline powder | | | | |
| Odor | No odor | No odor | No odor | No odor | No odor | No odor |
| D-allulose, g/100g; dry wt. basis | ≥98.0 | 99.62 | 99.90 | 99.73 | 99.63 | 99.42 |
| D-allulose, g/100g; as-is basis | ≥98.0 | 99.43 | 99.77 | 99.53 | 99.43 | 99.22 |
| Moisture, g/100g | <2.0 | 0.21 | 0.20 | 0.21 | 0.19 | 0.22 |
| Ash, g/100g | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Pb, mg/kg | ≤0.1 | ≤0.05 | ≤0.05 | ≤0.05 | ≤0.05 | ≤0.05 |
| As, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Cd, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Hg, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Total plate count, CFU/g | ≤1,000 | <1,000 | <1,000 | <1,000 | <1,000 | <1,000 |
| Coliforms, CFU/g | ND | ND | ND | ND | ND | ND |
| *Salmonella*, CFU/25 g | ND | ND | ND | ND | ND | ND |
| *Staphylococcus aureus*, CFU/g | ND | ND | ND | ND | ND | ND |

CFU=colony forming units; ND=not detected.

Table B.6.1-2. Specifications and Analytical Values of Product 2 (D-Allulose Syrup H)

| Composition | Specification | Lot Number | | | | |
|---|---|---|---|---|---|---|
| | | 2018 1003 | 2018 1115 | 2018 1212 | 2019 0409 | 2019 0826 |
| Appearance | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid |
| Odor | No odor | No odor | No odor | No odor | No odor | No odor |
| D-allulose, g/100g; dry wt. basis | ≥90 | 98.5 | 98.0 | 98.2 | 97.5 | 98.7 |
| D-allulose, g/100g; as-is basis | ≥62 | 74.8 | 74.2 | 74.2 | 74.1 | 73.8 |
| Non-allulose saccharides, g/100g; as-is basis | <6 | 1.1 | 1.5 | 1.2 | 2.1 | 0.8 |
| Moisture, g/100g | <32 | 24.1 | 24.3 | 24.6 | 23.8 | 25.4 |
| Ash, g/100g | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Pb, mg/kg | ≤0.1 | ≤0.05 | ≤0.05 | ≤0.05 | ≤0.05 | ≤0.05 |
| As, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Cd, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Hg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Total plate count, CFU/g | ≤1,000 | <1,000 | <1,000 | <1,000 | <1,000 | <1,000 |
| Coliforms, CFU/g | ND | ND | ND | ND | ND | ND |
| *Salmonella*, CFU/25 g | ND | ND | ND | ND | ND | ND |
| *Staphylococcus aureus*, CFU/g | ND | ND | ND | ND | ND | ND |

CFU=colony forming units; ND=not detected.

Table B.6.1-3. Specifications and Analytical Values of Product 1 (D-Allulose Syrup L)

| Composition | Specification | Lot Number | | | | |
|---|---|---|---|---|---|---|
| | | 2018 0920 | 2018 1102 | 2018 1220 | 2019 0115 | 2019 0226 |
| Appearance | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid |
| Odor | No odor | No odor | No odor | No odor | No odor | No odor |
| D-allulose, g/100g; dry wt. basis | ≥10 | 16.0 | 17.57 | 17.0 | 17.5 | 14.9 |
| D-allulose, g/100g; as-is basis | ≥8 | 12.2 | 13.4 | 13.0 | 13.3 | 11.4 |
| Non-allulose saccharides, g/100g; as-is basis | <67 | 64.3 | 62.7 | 63.3 | 62.9 | 65.3 |
| Moisture, g/100g | <25 | 23.5 | 23.9 | 23.7 | 23.8 | 23.3 |
| Ash, g/100g | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Pb, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| As, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Cd, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Hg, mg/kg | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 | ≤0.1 |
| Total plate count, CFU/g | ≤1,000 | <1,000 | <1,000 | <1,000 | <1,000 | <1,000 |
| Coliforms, CFU/g | ND | ND | ND | ND | ND | ND |
| *Salmonella*, CFU/25 g | ND | ND | ND | ND | ND | ND |
| *Staphylococcus aureus*, CFU/g | ND | ND | ND | ND | ND | ND |

CFU=colony forming units; ND=not detected

Table B.6.1-4 Carbohydrate Composition of Product 3 (D-Allulose Syrup L)

| Composition | COA (1 lot) | COA (2 lot) | COA (3 lot) | COA (4 lot) | COA (5 lot) | Mean |
|---|---|---|---|---|---|---|
| Appearance | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid | Clear yellow liquid |
| Odor | No odour | No odour | No odour | No odour | No odour | No odour |
| Allulose*, g/100g | 12.2 | 13.4 | 13.0 | 13.3 | 11.4 | 12.7 |
| Fructose*, g/100g | 35.3 | 34.3 | 34.6 | 34.7 | 37.0 | 35.2 |
| Glucose*, g/100g | 27.2 | 26.7 | 27.1 | 26.4 | 26.9 | 26.9 |
| Oligosaccharides*, g/100g | 1.8 | 1.7 | 1.6 | 1.7 | 1.4 | 1.6 |
| Moisture, g/100g | 23.5 | 23.9 | 23.7 | 23.8 | 23.3 | 23.6 |

*On an As-Is basis.

Table B.6.1-5 Residual mineral levels in D-allulose ingredients

| Product | Lot number | | | | |
|---|---|---|---|---|---|
| Crystalline powder | 2018 0516 | 20180716 | 20181202 | 20190228 | 20190515 |
| Mg, mg/kg | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 |
| Mn, mg/kg | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 |
| D-allulose syrup H | 20181003 | 20181115 | 20181212 | 20190409 | 20190826 |
| Mg, mg/kg | 0.173 | 0.212 | 0.167 | ≤0.07 | ≤0.07 |
| Mn, mg/kg | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 |
| D-allulose syrup L | 20180920 | 20181102 | 20181220 | 20190115 | 20190226 |
| Mg, mg/kg | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 |
| Mn, mg/kg | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 | ≤0.07 |

## B.7 Analytical method for detection of a novel food ingredient

*(addressing section 3.5.2.B.7 of the FSANZ Application Handbook)*

D-allulose content is detected via high-performance liquid chromatography (HPLC). HPLC and $^{13}C$ nuclear magnetic resonance (NMR) confirmed that the D-allulose manufactured by Samyang is chemically and structurally identical to those of the reference material (source, Carbosynth). Analysis has confirmed the chemical equivalence of Samyang's D-allulose to D-allulose reference source as follows:

HPLC analysis

To identify and analyse the purity of crystalline allulose, allulose samples were assayed by HPLC (model: Agilent 1260 Infinity system) analysis with the following conditions:

- Analysis column: Bio-rad Aminex HPX-87C column, 300 mm×7.8 mm
- (Catalog #125-0095 or equivalent)
- Mobile phase: Deionised water (100%)
- Flow rate: 0.6 mL/minute
- Column temperature: 80°C
- Detector: Refractive index (RI) detector

Similar retention times were observed for the main component (Samyang's D-allulose vs. the reference D-allulose: 20.61 vs. 20.67 minutes). The data indicate that Samyang's D-allulose mimics or is identical to the D-allulose reference (Carbosynth).

NMR analysis

Identity of the product was confirmed by comparison with the $^{13}C$ NMR spectra of Samyang's D-allulose to a reference sample purchased from Carbosynth (NMR spectrometer: Bruker AVANCE III HD 500; 125 MHz for $^{13}C$). Both the standard and Samyang's D-allulose (Lot20170701) were dissolved in deuterium oxide ($D_2O$) and analysed by $^{13}C$-NMR using a 125 MHz NMR spectrometer.

The chemical shifts of the $^{13}C$ resonances of the pyranose and furanose forms of the D-allulose standard and Samyang's sample showed that the samples from Samyang had the same spectrum as that of the allulose standard, which was correctly identified as D-allulose. Details are presented in Annex D.

Other components present in Samyang's D-allulose syrup

Samyang's D-allulose syrup (10%) also contains other mono- and oligosaccharides as identified by HPLC analysis: fructose (34 to 37%), glucose (26-28%), and oligosaccharides with degree of polymerisation unit of 2 to 5 (less than 1.8%).

## B.8 Technical information on the processing aid

*(addressing section 3.3.2.A of the FSANZ Application Handbook)*

### *B.8.1 Information on the type and identity of the processing aid*

*(addressing section 3.3.2.A.1 and 3.3.2.A.2 of the FSANZ Application Handbook)*

D-psicose 3-epimerase (alternative name: D-allulose 3-epimerase (EC 5.1.3.30)) is an enzyme of microbial origin that should be added to section S18—4(5) if approved for use as a processing aid. The enzyme is expressed in non-genetically modified *Microbacterium foliorum* (SYG27B-MF).

EC Tree

- 5 Isomerases
  - 5.1 Racemases and epimerases
    - 5.1.3 Acting on carbohydrates and derivatives
      - 5.1.3.30 D-psicose 3-epimerase

### *B.8.2 Information on the chemical and physical properties of the processing aid*

*(addressing section 3.3.2.A.3 of the FSANZ Application Handbook)*

The manufacturing process of Samyang's D-allulose described above clearly demonstrates that the enzyme produces D-allulose at the levels required to meet the specifications for both the syrup and powder varieties. The enzyme, and the organism that naturally harbours the enzyme, is not present in final D-allulose preparations and is therefore not present in D-allulose sold as a food or food ingredient.

D-psicose 3-epimerase is highly specific for D-allulose. The enzyme can be sourced from a variety of microorganisms. Some genetically modified source organisms have been the subject of international uses of D-allulose (see section 9.2.2 above). As noted above, Samyang produces D-allulose from the enzyme which is expressed in non-genetically modified *M. foliorum* SYG27B-MF.

The activity of D-psicose 3-epimerase is [redacted], where 'U' is the amount (mM) of allulose that is converted from fructose per gram of cells or enzymes. The number of cells per immobilised bead is [redacted] of beads. The optimal pH for enzyme activity is 6.5 at a temperature of 60°C. The enzyme's molecular weight is 31.4 kDa. The enzymatic epimerisation of fructose to D-allulose can be promoted in the presence of manganese ions (Mn(2+)). Samyang uses manganese sulphate as a source of manganese to promote the epimerisation process.

### *B.8.3 Manufacturing process*

*(addressing section 3.3.2.A.4 of the FSANZ Application Handbook)*

The manufacture of the processing aid, including the cultivation of *M. foliorum* SYG27B-MF and preparation of the immobilisation bead for D-allulose production is described in detail in Annex J (CCI). The manufacture of D-allulose from M. foliorum SYG27B-MF is described in detail in section B.5.1.

### *B.8.4 Specification for identity and purity*

*(addressing section 3.3.2.A.5 of the FSANZ Application Handbook)*

There is no specification for D-psicose 3-epimerase in Schedule 3 of the Code. There are international specifications for enzyme preparations used in food production. The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2017) has established specifications for enzyme preparations and these specifications arelisted in section S3—2 of the Code as a primary source of specifications for enzyme preparations that are permitted by the Code. Samyang's D-psicose 3-epimerase enzyme is not a purified enzyme preparation. The enzyme is naturally present in non-genetically modified *M. foliorum* SYG27B. Specifications for Samyang's M. foliorum SYG27B harbouring the enzyme D-psicose 3-epimerase meet the JECFA specifications for enzyme preparations. Specifications for M. foliorum SYG27B harbouring D-psicose 3-epimerase activity are presented in Table B.8.4-1.

Table B.8.4-1. Specifications and analysis value of *M. foliorum* SYG27B possessing D-psicose 3-epimerase activity the cells of the microbial cells obtained after culturing

| Composition | Unit | Specification | LOQ | Analysis Results | Analytical Method/ Reference |
|---|---|---|---|---|---|
| Appearance | - | Light yellow colour | - | Light yellow colour | Visual |
| Enzyme activity /g_*M. foliorum* DCW | U/g_DCW | [redacted] | | [redacted] | Internally validated method |
| *M. foliorum* cell counts /g_DCW | CFU/g_DCW | [redacted] | | [redacted] | Internally validated method |
| Protein /g_*M. foliorum*_DCW | % (wt/wt) | [redacted] | | [redacted] | AOAC 945.23 |
| Ash | % (wt/wt) | NA | - | 1.22 | AOAC 900.02 |
| Moisture | % (wt/wt) | [redacted] | - | [redacted] | AOAC 941.14 |
| TOS g/100 g *M. foliorum* CWW | % (wt/wt) | [redacted] | - | [redacted] | KF code |
| Hg | µg/kg | ≤100 | 50 | Not detected | KF Code, DMA |
| Pb | mg/kg | ≤0.3 | 0.05 | Not detected | AOAC 2015.01 /ICP-MS |
| Cd | mg/kg | ≤0.1 | 0.04 | Not detected | AOAC 2015.01 /ICP-MS |
| As | mg/kg | ≤0.1 | 0.05 | Not detected | AOAC 2015.01 /ICP-MS |
| Total plate count | CFU/g | ≤100 | - | 0 | AOAC 2002.07 |
| *Escherichia coli* | CFU/g | ≤10 | - | 0 | AOAC 991.14 |
| *Listeria monocytogenes* | Presence/25 g | Negative | - | Negative | AOAC 993.09 |
| *Salmonella* | Presence/25 g | Negative | - | Negative | AOAC 989.14 |
| Mold & yeast plate count | CFU/g | ≤20 | - | 0 | AOAC 997.02 |

CFU = colony forming unit; DCW = dry cell weight; LOQ = limit of quantitation; NA = not applicable; wt = weight, TOS = total organic solids

The enzyme activity and total organic solids content of five non-consecutive representative batches of *M. foliorum* SYG27B harbouring D-psicose 3-epimerase activity are presented below in Table B.8.4-2.

Table B.8.4-2. Compositional data of *M. foliorum* SYG27B possessing the food enzyme, D-psicose 3-epimerase from 5 non-consecutive batches

| Parameter, unit | Batch 1 | Batch 2 | Batch 3 | Batch 4 | Batch 5 | Average |
|---|---|---|---|---|---|---|
| D-psicose 3-epimerase activity/*M. foliorum*, U/g *M. foliorum* DCW | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] |
| *M. foliorum* cell counts, CFU/g DCW | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] |
| Protein/*M. foliorum* DCW, g/100 g *M. foliorum* DCW | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] |
| Ash, g/100 g *M. foliorum* CWW | 1.25 | 1.25 | 1.23 | 1.17 | 1.22 | **1.22** |
| Water, g/100 g *M. foliorum* CWW | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] |
| Total TOS, g/100 g *M. foliorum* CWW | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] | [redacted] |

%TOS calculated as 100% - % water - % ash, CFU = colony forming unit; CWW = cell wet weight; DCW = dry cell weight; TOS = total organic solid

## *B.8.5 Analytical method for detection*

*(addressing section 3.3.2.A.6 of the FSANZ Application Handbook)*

The enzyme and the *M. foliorum* organism harbouring the enzyme is not present in final D-allulose products that are sold as food or incorporated into foods as ingredients. As noted in Section B.4.2 above, Samyang has conducted testing to ensure no *M. foliorum* is present in commercial D-allulose products (in both the syrups and powder forms). No colony forming units were detected after 6 days of incubation of the syrup and powder products; nor was any DNA of *M. foliorum* detected in commercial D-allulose products after applying a primer that specifically acts on the production strain. More detail in provided in Annex E (CCI).

# C INFORMATION ON THE SAFETY OF THE NOVEL FOOD

*(addressing section 3.5.2.C of the FSANZ Application Handbook)*

As discussed in section B.1, D-allulose is a dietary macro-component. Guideline 3.5.2.C.4 of the FSANZ Application Handbook sets the same information requirements for dietary macro-components and single chemical entities. These requirements are addressed below.

## C.1 Single chemical entities and Dietary macro-components

*(addressing section 3.5.2.C.4 of the FSANZ Application Handbook)*

### *C.1.1 Information on the toxicokinetics and metabolism of the dietary macro-component and, where appropriate, its degradation products and major metabolites*

*(addressing section 3.5.2.C.4.1 of the FSANZ Application Handbook)*

#### *C.1.1.1 Animal and in-vitro studies*

Tsukamoto et al. (2014) evaluated the pharmacokinetics of D-allulose in Wistar rats using $^{14}C$-labeled D-allulose. After both oral and intravenous administrations, concentrations in whole blood, urine, and organs were measured at different time points until 2 hours and 7 days after a single oral administration (100 mg/kg bw) to Wistar rats. D-allulose is partly absorbed in the digestive tract and enters the bloodstream. Following oral administration, the maximum blood concentration (48.5±15.6 μg/g) was observed at 1 hour. Excretions via urine were 20% and 33% within 1 hour- and 2 hour-periods, respectively. Only 1% of $^{14}C$-labeled D-allulose remained in the whole body at 7 days after the single-dose oral administration. In addition, autoradiography was also performed by injecting 100 mg/kg bw of $^{14}C$-labeled D-allulose or glucose intravenously to C3H mice. Following intravenous administration, almost 50% of $^{14}C$-labeled D-allulose was excreted via urine within 1 hour. The half-life in blood was determined to be 57 minutes. Accumulation in organs was detected only in the liver.

Whistler et al. (1974) evaluated the metabolism of D-allulose in rats using D-[U-$^{14}C$] allulose and measured the contents in urine, feces, glycogen, exhaled carbon dioxide, and carcasses 6–72 hours after oral (20 mg, 2 uCi) and intravenous (15 mg, 1.5 uCi) administrations. Within 6-7 hours, urinary excretions were approximately 35% for orally administered D-allulose and 98% for intravenously administered D-allulose.

It appears that a single bolus dose may increase the rate of fecal excretion and cecal fermentation into short chain fatty acids (SCFAs). When D-allulose is orally administrated (5 g/kg bw) to Wistar rats, urinary and fecal excretions of D-allulose over 24 hours were 11-15% and 8-13% of dosage, respectively (Matsuo et al., 2003). The level tested in this study may correspond to a single bolus of 300 g D-allulose in humans.

Matsuo et al. (2003) reported the metabolic effects of D-allulose in rats. Four-week-old rats were fed a high carbohydrate diet, including 5% corn oil; 0, 10, 20 or 30% D-allulose; and 65, 55, 45 or 35% corn starch for 34 days. As a result, body weight gain, food intake and food intake were decreased in a dose dependent manner. For D-allulose doses of 0, 10, 20, or 30; respective calculated D-allulose intake was 0, 42.2, 73.4, or 78.3 g/34 days; respective body weight gains were 124, 101, 81, and 42 g/34 days; and respective cecal weights were 0.4, 0.64, 1.11 and 1.92 g. Continuous administration of D-allulose increased cecal SCFA, as D-allulose is

fermented in the cecum by intestinal microflora. Cecal SCFA content increased in a dose dependent manner as the dietary D-allulose level increased (control vs. 10 g vs. 20 g vs. 30 g: acetic acid, 5 vs. 7 vs. 12 vs. 23 mmol/cecum; propionic acid, 1.3 vs. 1.7 vs. 2.1 vs. 2.6 mmol/cecum; butyric acid, 1.0 vs. 3.0 vs. 5.5. vs. 8.5 mmol/cecum; all the values are approximate read from graphs, $P<0.05$ for control vs. most of test groups). Consumption of non-digestible carbohydrates are often associated with an increased cecal weight, which is not considered a toxicological concern (Leegwater et al., 1974).

Kishida et al. (2019) reported that d-allulose competed with D-[$^{14}$C]-fructose and the affinity of d-allulose for GLUT5 was lower than that of d-fructose. When d-allulose alone was gavaged, plasma d-allulose levels were dramatically higher in rats previously fed fructose. When d-allulose was gavaged with d-fructose, previously observed increases in plasma d-allulose levels were dampened and delayed, tracer D-[$^{14}$C]-fructose uptake rate was reduced to 54.8% in 50 mM d-allulose and to 16.4% in 50 mM d-fructose. The data indicated that d-fructose inhibited transepithelial d-allulose transport into plasma and that GLUT5 mediates intestinal d-allulose transport at lower affinity relative to d-fructose.

Hishiike et al. (2013) reported that D-allulose was absorbed similarly to fructose, using a Caco-2 cell monolayers model. Both D-allulose and fructose were absorbed from the intestinal lumen into enterocytes via GLUT5 (facilitated glucose transporter) and possibly GLUT7 and released to the lamina propria vita GLUT2. This differs from the uptake of glucose into small intestine epithelial cells, which is mediated mainly by a sodium dependent glucose cotransporter. Fructose has a high permeability and intestinal absorption, which suggests that D-allulose also has high intestinal absorption in the human intestine. Using the same Caco-2 cell monolayer model, Hishiike et al. also reported that the addition of multiple sugars (glucose, fructose and D-allulose) onto the Caco-2 monolayer reduced the permeability of each sugar by over one third when compared to the addition of individual sugars alone. This occurred when two sugars (glucose and fructose) were added together and when all three sugars were added together. The authors suggested it is probable that these sugars compete with one another at a sugar transporter on the basolateral membrane (such as GLUT2), noting that glucose absorption into enterocytes takes place via a different route to fructose and D-allulose.

Maeng et al. (2019) reported that D-allulose was stable for up to 240 minutes in phosphate-buffered saline, while glucose and fructose were not detected; suggesting D-allulose is not degraded under physiological or general assay conditions. Maeng et al. also examined the stability of D-allulose in simulated gastric fluid (SGF) containing pepsin and in fasted state simulated intestinal fluid (FaSSIF). D-allulose was stable in both mediums for 60 and 240 minutes respectively, indicating D-allulose is not sensitive to pH or enzymatic degradation in the gastrointestinal tract. D-allulose was also reported to be metabolically stable in human and rat hepatocytes after 240 minutes, suggesting minimal metabolism of D-allulose in the liver. D-allulose was also shown to have negligible effect on the metabolisation of glucose and fructose by hepatocytes (both human and rat). Fructose was evaluated separately in the same manner as D-allulose, with percentages of fructose remaining in human and rat hepatocytes markedly decreased after 240 minutes. This is in keeping with previous reports of the liver being the organ that predominately extracts fructose. Erythritol was similarly evaluated as a reference compound, recognising it is known as a zero-energy sugar alcohol. Erythritol was stable for up to 240 minutes in human and rat hepatocytes, indicating minimal metabolism in the liver; as reported for D-allulose.

Maeng et al. also evaluated plasma concentration time profiles of intravenously administered D-allulose in rats for time periods up to 240 minutes. D-allulose was rapidly eliminated from plasma with a mean half-life and total body clearance of 72.2 minutes and 15.8 mL/min/kg, respectively. The authors suggest that given the stability of D-allulose in rat hepatocytes, the rapid elimination from the systemic circulation was probably due to elimination via the renal route, which has been reported in other studies (Tsukamoto et al. (2014) and Matsuo et al. (2003)).

### *C.1.1.2 Human studies*

Human studies have observed that most orally administered D-allulose is absorbed and excreted intact in urine. The small amount of D-allulose passing to the large intestine is poorly fermented and excreted in faeces if not fermented. Metabolism of D-allulose is similar to that of erythritol, which is quickly absorbed and excreted via urine.

Tate & Lyle commissioned a mass balance study using a radioisotope label ($^{14}C$) of allulose to determine absorption, excretion and fermentability of allulose (Williamson et al. 2014, unpublished). The study by Williamson et al. has not been published but the results of the study have been communicated by Tate & Lyle and assessed by the FDA in the context of investigating label declarations of D-allulose in the US (see section B.2 above).

Eight healthy adult males received a single dose of a 240 mL solution containing 15 g allulose and 776 nCi of $^{14}C$(U)-allulose after a light breakfast. Blood plasma, urine and faecal samples were collected after administration for up to 168 hours for analysis. Plasma total radioactivity recovery demonstrated that the $^{14}C$ radiotracer was rapidly absorbed. The average peak plasma concentration occurred within 1 hour of administration. Within 24 hours, the majority of the $^{14}C$ radiotracer was cleared from the plasma. Urine and faecal samples demonstrated that urine was the major route of elimination (84-93% of recovered $^{14}C$ radiotracer) while faeces was a minor route (2-6%). One subject exhibited very low recovery in the urine sample and was excluded. Within 48 hours, majority of the radiotracer was eliminated via urine and faeces.

Table C.1.1-1. $^{14}C$ Radiotracer Mass Balance in Eight Humans*

| | % of Dose Recovered |
|---|---|
| Faecal | 3.12 ± 1.23 |
| Urine | 81.47 ± 13.92 |
| Total | 84.59 ± 15.14 |

*Adopted from Tate and Lyle

For radioactive component, urine was the most dominant route of elimination. Allulose was identified as the predominant compound in urine. In urine and feces, allulose was detected at 70.4% of the original dose. In plasma, allulose was recovered at 80.3% of total radioactivity.

Expired air samples for $^{14}CO_2$ detection were collected prior to and after administration at selected intervals up to 6 hours. Results of the expired air recovery suggest that D-allulose that passed to the large intestine was poorly fermented with minimal detection of $^{14}C$ in breath samples (94% of samples below detection levels (lower limit of quantification of <50 dpm with a maximum of 79 dpm). No adverse events were reported. The product was well tolerated in healthy adult males.

Iida et al. (2010) used four trials measuring breath and urine after consumption of various doses of allulose (and controls). The first trial calculated carbohydrate energy expenditure (CEE) in six adult subjects after oral administration of D-allulose compared to starch hydrolysate (both at 0.35 g/kg bw) and water. Using the Weir method, respiratory exchange (carbon dioxide and oxygen) was measured for 180 minutes after administration and urinary nitrogen was measured at the end of the 180-minute measurement period. After starch hydrolysate ingestion, CEE increased at around 30 minutes, peaking at 60-90 minutes. In contrast, no increase in CEE was measured after D-allulose or water ingestion. These results suggest that D-allulose is not metabolised into energy when consumed at a typical dose (0.35 g/kg bw - approximately 20 grams for a 60 kg person in this study).

The second and third studies investigated D-allulose excretion in urine and fermentability in the large intestine. Fourteen subjects received doses of D-allulose or fructooligosaccharide (FOS) at 0.33 g/kg bw (corresponding to 20 g for 60 kg subject – D20), 0.17 g/kg BW (10 g – D10) or 0.08 g/kg bw (5 g – D5). Urine was collected 12 hours after ingestion and again at 24 and 48 hours (study 2). Breath hydrogen collection occurred before ingestion and hourly after ingestion for 10 hours (study 3). D-allulose urinary excretion rates after 48 hours were 66.2% ± 12.6% for D20, 78.6% ± 10.6% for D10 and 78.8% ± 11.7% for D5. The majority of D-allulose excreted in urine for each dose was excreted in the first 12 hours (80-82% of 48-hour cumulative excretion was in first 12 hours).

Study 3 investigated the fermentation of the D-allulose that was not absorbed in the small intestine and passed to the large intestine via hydrogen breath analysis. This study was performed to estimate an energy value for D-allulose by comparing the results against FOS, which is known to have an energy value of 8.4 kJ/g (the energy value component is discussed in more detail in section B.2 above). The area under the curve (AUC) for excreted hydrogen gas was significantly lower for D-allulose than FOS, suggesting that minimal D-allulose is fermented in the large intestine:

AUC: F20 = 506.9 ± 437.5 ppm/h; D20 = 56.0 ± 91.0 ppm/h
AUC: F10 = 291.0 ± 227.5 ppm/h; D10 = 26.7 ± 33.7 ppm/h
AUC: F5 = 148 ± 151.1 ppm/h; D5 = 25.3 ± 37.6 ppm/h

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

Study 4 investigated D-allulose fermentability in the large intestine after an adaptation period. Eight subjects ingested 5 g of D-allulose 3 times a day for 8 weeks. End-expiratory gas collection was conducted on the first and last day of the ingestion period. There was no statistically significant difference in breath hydrogen excretion after D-allulose ingestion before and after the adaptation period, suggesting that there is no adaptation by intestinal microflora with respect to fermentation of D-allulose. In addition, Iida et al. tested fermentability of D-allulose by intestinal bacteria by inoculating 35 intestinal bacteria strains. D-allulose fermentability was not identified in 31 of the 35 strains, with the other four strains showing low fermentability.

In summary, the data consistently demonstrated that D-allulose was rapidly absorbed in the small intestine and the unchanged intact molecule was excreted rapidly via urine. D-allulose, which reached the large intestine, was not fermented in the large intestine.

*C.1.1.3 Metabolic fate and gastrointestinal tolerance of erythritol in humans*

Erythritol is a food additive approved by FSANZ for widespread use in foods at levels consistent with good manufacturing practice. D-allulose and erythritol have similarities in metabolic fates as follows:

1) Both D-allulose and erythritol are rapidly absorbed;

2) A significant portion of erythritol is excreted in urine unmetabolised. In animals and humans, depending on dose, 60-90% of ingested erythritol is rapidly absorbed from the small intestine and excreted unchanged in the urine (from GRN 208);

3) Both have calorie values of 1.6 kJ/g or less; and

4) If part of these substances escapes the absorption in the upper gastrointestinal tract, they may not be metabolised by fecal flora. Unabsorbed erythritol is fermented to SCFA in the colon (from GRN 208) or is excreted in the feces.

Thus, metabolic fate of erythritol is briefly reviewed in this section.

Noda et al. (1994) investigated the effect of an oral administration of erythritol on serum glucose and insulin levels, and estimated the available energy of erythritol in five healthy male volunteers, aged 45-58 years. Serum levels of erythritol reached the maximum concentration of 426.5 μg/mL at 30 min and gradually declined to 13.5 μg/mL at 24 hours. Total urinary excretion of erythritol was 85.8 ± 4.6% for 24 hours and 90.3 ± 4.5% for 48 hours. Erythritol did not significantly change the serum concentrations of glucose or insulin, although the same dose of glucose rapidly increased the serum glucose and insulin levels within 30 minutes post administration. Erythritol did not induce any significant effects on serum levels of total cholesterol (TC), triglycerides (TG), free fatty acids, and electrolytes, including Na, K, and Cl, as well as urinary concentrations of electrolytes. Noda et al. (1994) also reported that mean plasma glucose and insulin levels were unaffected by erythritol in the 3-hour period post administration. The results of this study indicate that erythritol was readily absorbed following oral administration and was excreted unchanged in the urine. Less than 20% of erythritol remained unabsorbed and was available for colonic fermentation and potential production of short chain fatty acids (SCFAs). Its caloric value was estimated to be 1.7 kJ/g or less[3].

The subsequent study by Noda et al. (1996) confirmed the previous findings that D-erythritol is quickly absorbed and excreted via urine. After a single oral administration of radiolabeled erythritol in doses ranging from 0.125 to 2.0 g erythritol/kg bw, erythritol concentrations in the blood and plasma of rats reached their maxima 1 hour after administration and then declined biexponentially. At 8, 24, and 120 hours after administration, 44.4, 94.0, and 95.7% of the radioactivity had been excreted in the urine of dogs, respectively. In rats, the mean percentage from urinary excretion was 75.3% at 8 hours, 91.0% at 24 hours, and 92.7% at 120 hours after administration. Fecal excretions of the radioactivity were low: 0.33 and 1.19% of the radioactivity were recovered in dogs and rats, respectively. Only 1.17% had been excreted in expired air from dogs and 4.80% in expired air from rats. Other than stomach and gastrointestinal tract, liver and kidney were primary sites for distribution. Concentration of erythritol in liver and kidneys peaked at 0.5- and 1-hour post-administration, respectively.

[3] Erythritol has an energy factor of 1 kJ/g listed in Schedule 11 of the Code

Bornet et al. (1996) studied the metabolic fate of erythritol after a single oral dose administration of 1 g erythritol/kg bw in human volunteers. The plasma level of erythritol increased during the first 30 to 40 minutes, reaching a maximum value of approximately 2.2 mg/mL after 90 minutes, followed a tendency toward gradual decline to approximately 1.5 to 1.7 mg/mL at 3 hours post administration. Urine erythritol levels reached a maximum during the period from 60 to 120 minutes after ingestion. Approximately 30% and 78% of the ingested amount of erythritol was excreted unchanged in the urine during the first 3 and 24 hours, respectively. Renal clearance of erythritol (62.0 mL/minute) was approximately half that of creatinine (120.2 mL/minute), indicating tubular reabsorption of erythritol by the kidney.

Consistent with findings of other studies, Hiele et al. (1993) also reported that a cumulative urinary recovery of 52.2% and 84.1% of the administered dose of erythritol during the first 6 and 24 hours, respectively. In this study, the metabolism of erythritol was assessed in six normal volunteers by measuring the amount of $^{13}CO_2$ excretion and $H_2$ excretion in breath, and erythritol in urine after intake of 25 g $^{13}C$-labelled erythritol. In addition, the $H_2$ production by fecal flora supplemented with small amounts of erythritol, glucose, and lactitol was measured *in vitro*, as an index of bacterial metabolism of non-absorbed substrate. Erythritol consumption did not induce increase in breath $^{13}CO_2$ and $H_2$, and erythritol was nearly completely recovered in urine.

*In vitro* experiments (Hiele et al., 1993) showed that no $H_2$ was formed by fecal flora from erythritol as compared with glucose and lactitol.

In summary, 78-90% of ingested erythritol is readily absorbed and excreted in urine without degradation, and its available energy in humans is less than 1.7 kJ/g (0.4 kcal/g) (Noda et al., 1994; Bornet et al. 1996). It is concluded that the metabolic fates of D-allulose and erythritol are similar: they are readily absorbed and undergo no metabolism by the host. If part of D-allulose or erythritol escapes absorption, they may or may not be metabolised by the fecal flora.

*C.1.2 Information from studies in animals or humans that is relevant to the toxicity of the dietary macro-component and, where appropriate, its degradation products and major metabolites*

*(addressing section 3.5.2.C.4.2 of the FSANZ Application Handbook)*

The safety of D-allulose has been extensively studied in animal and human experiments. Samyang Corp. has commissioned toxicology and human clinical studies on its D-alluose product. A summary of these studies and studies performed on other preparations of D-allulose are included below. The literature demonstrates D-allulose is well tolerated in animals and humans with transient gastrointestinal disturbance being the adverse effect associated with very high doses; a common observance with carbohydrates that are not metabolised in the body (including dietary fibres).

In addition to the data from Samyang's sub-chronic toxicity study in rats, the metabolism and safety data and other pertinent information discussed for other D-allulose preparations (GRN 400; GRN 498; GRN 693) are applicable to the safety of Samyang's D-allulose in this novel food application. It is due to the fact that the specifications for the powder form of D-allulose in this submission are similar to those described for other sources of D-allulose.

Table C.1.2-1 presents the summary of toxicity studies of Samyang's D-allulose (An et al., 2019) and other sources of D-allulose in animals. From a 90-day sub-chronic toxicity study of Samyang's D-allulose, the NOAEL was determined as 5,000 mg/kg bw/day, the highest dose tested.

A sub-chronic toxicity study (3-6 months) and a chronic toxicity study of another source of D-allulose (manufactured by Matsutano Chemicals) reported the NOAEL as 3% of the diet, the highest level tested (Matsuo et al., 2012; Yagi and Matsuo, 2009). In a subacute toxicity study in young rats, the NOAEL was determined to be up to 20% in the diet (corresponding to 10,000 mg/kg bw/day) (Matsuo et al., 2002b). In summary, D-allulose, like other monosaccharides, belongs to the group that has the lowest toxicity rating and is considered as an ordinary carbohydrate substance. Thus, the use of D-allulose in foods and beverages is not expected to pose a safety concern.

The $LD_{50}$ value of D-allulose, 15.8-16.3 g/kg bw, is comparable to that of other monosaccharides, such as fructose (14.7 g/kg bw) and erythritol (15.3 g/kg bw). A compound, which has a $LD_{50}$ value of >15 g/kg bw in rats, is classified as 'relatively harmless' (Altug, 2003).

Table C.1.2-1. Summary of sub-chronic and chronic toxicity studies of D-allulose in animals

| Species | Dosage | Duration | Primary endpoints and NOAEL | Reference |
|---|---|---|---|---|
| Samyang's D-allulose Produced by Non-GMO *M. foliorum* | | | | |
| Rats, SD | 0, 1,250, 2,500, and 5,000 mg/kg bw | 90 d | NOAEL- 5,000 mg/kg bw/day, the highest level tested | An et al., 2019 |
| Other Sources of D-allulose | | | | |
| Male rat | 8, 11, 14, 17, and 20 g/kg | Single dose | Acute toxicity: $LD_{50}$ was 6.3 g/kg bw | Matsuo et al., 2002a |
| Young rats | 10, 20, 30, and 40% in the diet | 34 days | Feed intake, body wt gain, and organ wt; NOAE= not determined by the author | Matsuo et al., 2002a |
| Dogs, beagle | 0 or 200 mg/kg bw | 12 weeks | Clinical biochemistry, hematology, bw NOAEL- 200 mg/kg bw/d | Nishii et al., 2017 |
| Diabetic rats* | 5% in the diet | 60 weeks | Fasting blood glucose, insulin, $HbA_{1c}$, lipid profiles, organ weight, and body fat composition including adipose tissue; NOAEL= 5% in the diet | Hossain et al., 2015a, 2015b |
| 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.
*Type 2 diabetes mellitus (T2DM) model Otsuka Long-Evans Tokushima Fatty (OLETF) was used for diabetic rats.

*C.1.2.1 Sub-chronic Toxicity*

*C.1.2.1.1 Sub chronic oral toxicity study of Samyang's D-allulose*

A 90-day sub chronic toxicity study of Samyang's D-allulose was conducted to evaluate the toxicity of repeated administration of D-allulose (An et al. 2019). The test substance was orally administered to Sprague-Dawley rats (10 male and 10 female rats) at a dose of 1250, 2500, or 5000 mg/kg for 90 days. Observed or analysed toxicological indicators included clinical signs, body weight, feed intake measurement, eye test, urinalysis, blood serum chemistry, hematology, and absolute and relative organ weights. In addition, gross necropsy findings and histopathological examinations were performed. None of the animals died during the period of administration, and there were no treatment-related abnormalities in any parameters tested. Based on the results of the 90-day repeated oral toxicity test of D-allulose, the NOAEL in Sprague-Dawley rats (males and females) was determined to be 5000 mg/kg/day, the highest level tested. Details are summarised below. The full study report supporting An et al. (2019) is available at Annex L (CCI).

1) Clinical signs and mortality

There were no deaths during the test period in all groups including the control group. As a result of the observation of clinical signs during the period of dosing, animal with abnormalities was not observed at all groups.

2) Body weight

A statistically significant decrease (11.9%) in body weight was found in the male high dose group compared to male control group. However, it was considered as a minor D-allulose related change.

3) Food consumption

The average intake of feed was not significantly different between the test group and the control group.

4) Urinalysis

Urine analysis was completed on week 13 of administration. There were no toxicologically relevant or statistically significant differences in urinalysis parameters noted during the study.

5) Hematology

Tested hematology parameters included white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), mean corpuscular Hb concentration, red cell distribution width, Hb distribution width, reticulocyte, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells.

Male mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular Hb concentration (MCHC) were significantly decreased in the high-dose group compared to the control group ($P<0.05$). However, the values were determined to be non-compound-related changes as they were within the normal range (historical data). Male red cell distribution width (RDW) was significantly increased in the high-dose group compared to the control group ($P<0.05$). However, the values were determined to be non-compound-related changes as they were within the normal range (historical data). Male neutrophil (NEU: 103/μL) was significantly decreased in the low- and high-dose groups compared to the control group ($P<0.05$).

However, the values were determined to be non-compound-related changes as they were within the normal range (historical data).

Female hemoglobin concentration (HGB) was significantly decreased in the high-dose group compared to the control group (P<0.05). However, the values were determined to be non-compound-related changes as they were within the normal range (historical data). Female monocyte (Monocyte) was significantly decreased in the medium-dose group compared to the control group (P<0.05). However, the values were determined to be non-compound-related changes as they were within the normal range (historical data).

Additionally, there were no abnormalities in the blood coagulation in the test groups compared to the control group.

6) Blood serum chemistry

Blood serum chemistry parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, glucose, total cholesterol, protein, creatine phosphokinase, albumin, bilirubin, triglyceride, inorganic phosphorus, albumin/globulin ratio, and calcium ion.

Statistically significant differences from control were detected for several clinical chemistry parameters. Test substance-related changes in clinical chemistry parameters were limited to an increase in ALP at medium (39.7%) and high dose (96.3%) levels as well as AST, total cholesterol (CHO), and sodium ion for males with values approaching or below the historical control ranges. Blood urea nitrogen (BUN) were significantly increased in the male low dose group with values within historical control range. Thus, these differences were not considered as a toxicological concern.

7) Organ Weights, Gross Necropsy Findings, and Histopathological Examinations

The following organs were extracted during necropsy and weighed: adrenal gland, pituitary, ovaries, prostate gland, testes, epididymides, spleen, kidneys, heart, lungs, brain, and liver. For the weighed organs, relative weight compared to the weight measured at necropsy was calculated. The following organs were subjected to histopathological examinations: testes, epididymides, ovaries, uterus, vagina, bladder, spleen, stomach, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph nodes, adrenal glands, kidneys, liver, femurs, submandibular lymph nodes, salivary glands, sternum, thymus, heart, lungs, aorta, tongue, trachea, esophagus, thyroid gland, eyes, Harderian gland, brain, pituitary gland, and skin (mammary gland).

Relative weight of the left epididymis in males was significantly increased in the medium- and high-dose groups compared with the control group (P<0.05). Relative weight of the brain in males was significantly increased in the low- and high-dose groups compared with the control group (P<0.05). Absolute and relative organ weight of the thymus in males was decreased in the high-dose group compared with the control group (P<0.05). However, these were determined to be non-compound-related changes because the values were within the normal range in the historical data. In addition, weight gain in the testis was interpreted as a minor compound-related change because they were within the normal range in the historical data.

Relative weight of the liver in males was significantly increased in the high-dose group (15.0%) compared with the control group (P<0.05). Relative weight of both kidneys in males was significantly increased in the high-dose group (Lt. 17.0%; Rt. 16.1%) compared with the control group (P<0.05). Absolute and relative organ weight of the liver in females was significantly

increased in the high-dose group (relative organ weight 18.5%) compared with the control group (P<0.05). Relative organ weight of both kidneys in females was significantly increased in the high-dose group (relative organ weight: Lt. 16.9%; Rt. 17.9%) compared with the control group (P<0.05). These results were identified as a minor compound-related change because they were within the normal range in historical data.

There were only occasional occurrences of gross necropsy and histopathological findings. These effects were considered to be non-compound related.

Summary

No significant differences were found in food intake, clinical signs, and mortality among the four groups. Compared to the control group, the high dose group had a 11.9% lower body weights, probably due to a lower energy intake associated with D-allulose. D-Allulose has a caloric value of 0.2 kcal/g. Thus, it was not considered as a toxicological concern. Significant changes in clinical chemistry and hematology parameters were not considered as toxicological concerns since the values were within the normal historical ranges. Significant changes in organ weights observed in some organs were not considered as toxicological concerns since these changes were not associated with any abnormalities from necropsy findings and/or histopathological examinations. There were only occasional, incidental occurrences of gross necropsy and histopathological findings. These effects were considered to be incidental and not treatment related. According to the test toxicity guidelines, NOAEL of Samyang's D-allulose was determined to be 5000 mg/kg/day in both male and female rats, the highest dose tested.

The histopathological test revealed lesions in the ovary, prostate, and stomach of the high-concentration group (Table C.1.2.1.1-1), but it was determined to be an individual-specific phenomenon or a spontaneous lesion which was also found in the control group. Therefore, there were no target organs by the substance to be inspected in this test.

Table C.1.2.1.1-1. Histopathological Results

| Histopathological Test Parameters | Groups | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | G1 (control) | G4 (high dose) | G1 (control) | G4 (high dose) |
| Ovary cysts | | | | |
| Minimal | | | - | 1/10 (2401) |
| Slight | | | - | - |
| Moderate | | | - | - |
| Severe | | | - | - |
| Prostate – Inflammatory cells infiltration | | | | |
| Minimal | 1/10 (1105) | 2/10 (1404, 1405) | | |
| Slight | - | - | | |
| Moderate | - | - | | |
| Severe | - | - | | |
| Kidney – Nephropathy | | | | |
| Minimal | 1/10 (1104) | - | - | - |
| Slight | - | - | - | - |
| Moderate | - | - | - | - |
| Severe | - | - | - | - |
| Liver – Focal necrosis of hepatocytes | | | | |
| Minimal | 1/10 (1109) | - | - | - |
| Slight | - | - | - | - |
| Moderate | - | - | - | - |
| Severe | - | - | - | - |

| Histopathological Test Parameters | Groups | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | G1 (control) | G4 (high dose) | G1 (control) | G4 (high dose) |
| Heart – Inflammatory cells infiltration | | | | |
| Minimal | 1/10 (1104) | - | - | - |
| Slight | - | - | - | - |
| Moderate | - | - | - | - |
| Severe | - | - | - | - |
| Stomach – Inflammatory cells infiltration | | | | |
| Minimal | - | 1/10 (1408) | 1/10 (2104) | - |
| Slight | - | - | - | - |
| Moderate | - | - | - | - |
| Severe | - | - | - | - |

*C.1.2.1.2 Acute and sub-acute toxicity studies of D-allulose*

In an acute administration test (Matsuo et al., 2002a), five groups of 8 male Wistar rats (3 weeks old) were orally given D-psicose in doses of 8, 11, 14, 17, and 20 g/kg bw. Three rats receiving 14 g/kg, three rats receiving 17 g/kg bw, and eight rats receiving 20 g/kg bw of D-psicose died within 2 days after administration. The calculated $LD_{50}$ values were 16.3 g/kg bw by the Behrens-Karber method and 15.8 g/kg bw by the Litchfield-Wilcoxon method. The $LD_{50}$ value of psicose is comparable to those of fructose (14.7 g/kg bw) and erythritol (15.3 g/kg bw). A compound that has a $LD_{50}$ value of 5 g/kg bw or higher in rats is classified as 'practically non-toxic' and a compound with a $LD_{50}$ value of 15 g/kg bw or higher as 'relatively harmless' (Altug, 2003). Psicose, 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-psicose in foods and beverages is not expected to pose a safety concern.

Matsuo et al. (2002b) studied the effects of a sub-chronic feeding of several levels of D-allulose in rats. Male Wistar rats (3-week-old) were fed diets containing 0 (control), 10, 20, 30, or 40% for 34 days. One rat fed 30% D-allulose diet and five rats fed 40% D-allulose diet died during the experimental period. Body weight gain, food intake, and food efficiency were more extensively suppressed by the higher D-allulose diets. The weights of heart, spleen, and abdominal adipose tissue were smaller in the order of dietary D-allulose concentration. Cecal hypertrophy was observed in rats fed 10-40% D-allulose diets. The feeding of diets extremely high in D-allulose seems to be harmful to the intestinal tract.

The results suggested that a large portion of D-allulose was not absorbed and fermented in the colon like other non-digestible carbohydrates in diets containing more than 10% D-allulose. Serum concentrations of glucose and TG were significantly lower in the 30% D-allulose group, but liver TG concentrations were significantly higher in the 10% D-allulose group. The relative liver and kidney weights were higher in the D-allulose groups than the control group. The authors related this phenomenon to that of D-tagatose, which increased the liver weight at dietary levels of 5 to 20% with histopathological alteration, because unabsorbed ketohexose tends to increase liver weight. The authors did not determine the NOAEL value from this study.

*C.1.2.1.2 Sub-chronic studies of other sources of D-allulose*

<u>Sub-chronic toxicity study in dogs</u>
Nishii et al. (2017) studied the safety and biological effects of D-allulose in healthy dogs. For 12 weeks, the dogs were administered D-allulose (0.2 g/kg bw) or placebo daily. Administration of

D-allulose at the dose rate of 200 mg/kg/day was well tolerated in dogs. D-Allulose administration did not influence clinical signs, body weight, hematological, or blood biochemical indices, except for total cholesterol concentrations which were decreased by 24% after 12 weeks. Blood biochemical tests included liver function enzymes (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase), lipid and glucose metabolism profile, urea nitrogen, bilirubin, and electrolytes. The authors concluded that the administration of D-allulose caused no harmful effects in dogs.

Sub-chronic toxicity study in rodents

A 90-day sub-chronic toxicity study reported the no observed adverse effect level (NOAEL) for D-allulose (manufacturer-Matsutani) as 3% of the 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% in the diet (or 1,280 mg/kg bw/day), the highest level tested, did not show adverse effects (Yagi and Matsuo, 2009).

Matsuo et al. (2002b) studied the effects of a sub-chronic feeding of several levels of D-allulose, a C3-epimer of D-fructose, in rats. Male Wistar rats (3-week-old) were fed diets containing 0 (control), 10, 20, 30, or 40% for 34 days. One rat fed 30% D-allulose diet and five rats fed 40% D-allulose diet died during the experimental period. Body weight gain, food intake, and food efficiency were more extensively suppressed by the higher D-allulose diets. The weights of heart, spleen, and abdominal adipose tissue were smaller in the order of dietary D-allulose concentration. Cecal hypertrophy was observed in rats fed 10-40% D-allulose diets. The feeding of diets extremely high in D-allulose seems to be harmful to the intestinal tract.

*C.1.2.2 Chronic toxicity and carcinogenicity studies*

*C.1.2.2.1 Chronic toxicity study of D-allulose by Yagi and Matsuo (2009)*

Yagi and Matsuo (2009) studied the long-term toxicity of D-allulose in male Wistar rats (3 weeks old) fed diets containing 3% D-allulose (or 1,280 mg/kg bw/day) or 3% sucrose (1,220 mg/kg bw/day) for 12-18 months. Body weight gain and intra-abdominal adipose tissue weight of rats fed the D-allulose diet for 18 months were significantly lower than those in rats fed the sucrose diet. Relative weights of liver and kidney were significantly higher in the D-allulose group than in the sucrose group, but it was not considered to be of toxicological significance. Rather, it is due to dietary D-allulose decreasing body fat accumulation and increasing liver glycogen as a consequence of serum glucose declining and serum insulin elevating. Increased relative weight of liver has been observed in animals fed other type of sugars, such as fructose and D-tagatose (GRN 352).

Age-related naturally occurring lesions were observed in the liver and kidneys at 12 months, but no abnormality due to ingestion of D-allulose was observed. Histopathologic observation of the liver at 18 months revealed fatty degeneration, and hepatocellular fibrosis were observed in the D-allulose group and not in the sucrose group. These findings tended to be slight and local. Histopathological observation at 12 months showed no difference in total pathological lesions between the sucrose and the D-allulose groups (liver, 4.13 vs. 3.13; kidney, 12.3 vs. 14.1, mean scores). At 18 months, the mean value for pathological lesions in the liver was significantly higher in the D-allulose group than in the sucrose group (2.75 vs. 3.75, mean scores), but the difference was slight ($P<0.05$). At 18 months, the total value for pathological lesions in the kidneys did not differ between the sucrose and the D-allulose groups (14.0 vs. 14.1, mean scores).

In this study, general hematology or serum chemistry tests were in the normal ranges. All values related to serum chemistry did not differ between the sucrose and D-allulose groups. Mean corpuscular hemoglobin at 12 months was significantly lower in the D-allulose group than in the sucrose group, but no differences were observed in any of the related hematology values. Hemoglobin and mean corpuscular volume at 18 months were significantly greater in the D-allulose group than in the sucrose group, but no differences were observed in any of the related hematology values. The histopathological data demonstrated that there were no toxicologically significant findings in rats given D-allulose at levels of 3% in the diet for 12-18 months. No gross pathological findings were evident at dietary doses of 3% D-allulose. The authors concluded that administration of D-allulose at 3% in the diet (or 1,280 mg/kg bw/day) did not result in any adverse effects in rats.

*C.1.2.2.2 A Chronic Study in Diabetic Rats by Hossain et al. (2015a, 2015b)*

Long-term administration (60 weeks) of D-allulose at a dose of 5% of the diet prevented and delayed the commencement and progression of type 2 diabetes through the control of blood glucose levels and postprandial hyperglycemia in diabetic rats (Hossain et al., 2015a, 2015b). 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 diabetic control group, the glucose levels started to increase slowly from 25 weeks and then sharply until 60 weeks, whereas in the diabetic allulose group, the glucose levels started to increase slightly from 45 weeks and remained constant until 60 weeks. By the end of the 60 weeks, fasting blood levels of glucose and glycosylated hemoglobin ($HbA_{1c}$) were decreased by approximately 40% in the diabetic allulose group compared to the diabetic control group (5% D-allulose in diabetic rats vs. 0% diabetic control vs. 0% non-diabetic control: $HbA_{1c}$, 3.3 vs. 5.0 vs. 1.5%; fasting blood glucose, 120 vs. 200 vs. 70 mg/mL, $P<0.05$ for each comparison with the allulose 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. Both fasting plasma low density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) concentrations decreased in the D-allulose diabetic group. No adverse effects of D-allulose were reported.

In summary, D-allulose supplementation in diabetic rats significantly improved glucose metabolism by controlling body weights, inflammatory biomarkers, fasting blood glucose, insulin, and $HbA_{1c}$ levels. No adverse effects of D-allulose were observed in glucose and lipid metabolism indicators.

*C.1.2.2.3 Conclusion from chronic toxicity studies*

Chronic toxicity studies showed a NOAEL of 1,280 mg/kg bw/day, the highest level tested. Samyang believes that chronic toxicity and carcinogenicity studies on Samyang's D-allulose (produced via non-GMM) are not necessary due to the following reasons:

1) Sub chronic toxicity showed no treatment-related abnormalities of D-allulose produced by a non-GMO in any parameters tested; food intake, body weights, clinical chemistry, hematology, absolute and relative weights of organs, and histological examination of organs as well as urine parameters. In addition, *in vitro* or *in vivo* toxicity tests, including genotoxicity tests, indicate that D-allulose is not mutagenic or genotoxic. Thus, carcinogenicity is not expected. D-Allulose is a monosaccharide. No monosaccharides have been recognised as carcinogens;

2) D-Allulose is rapidly excreted in the urine, and no significant amount of D-allulose is absorbed in the body to be used as an energy source;
3) A 12- to 18-month chronic toxicity study showed that another source of D-allulose (Matsutani Chemicals) did not show adverse effects at the dose of 3% in the diet (or 1,280 mg/kg bw/day), the highest level tested (Yagi and Matsuo, 2009); and
4) An efficacy study evaluation of a 60-week administration of D-allulose (Otsuka Pharmaceutical Co., Tokushima, Japan) at a dose of 5% of the diet reported no adverse effects on glucose metabolism (Hossain et al., 2015a, 2015b).

Due to substantial equivalence between Matsutani Chemicals' and Samyang's D-allulose in specifications (i.e., purity), the results found in the studies by Yagi and Matsuo (2009) can be applied when evaluating the safety of Samyang's D-allulose.

### *C.1.2.3 Reproductive and Developmental Toxicity and Teratogenicity Studies*

Samyang's D-allulose has been evaluated with respect to potential effects on reproductive and developmental parameters. No effects on reproductive or developmental parameters were reported at doses of D-allulose providing up to approximately 5,000 mg/kg body weight/day. A summary of relevant studies is provided in Table C.1.2.3-1.

Table C.1.2.3-1. Summary of Reproductive and Developmental Study and Teratogenicity Study of Samyang's D-allulose in Animals

| Species | Dosage | Duration | Primary endpoints and NOAEL | Reference |
|---|---|---|---|---|
| Reproductive and Developmental Study | | | | |
| Rats, SD | 0, 500, 1000 and 2000 mg/kg bw | Males 10 weeks Females 5 weeks | NOAEL- 2,000 mg/kg bw/day, the highest level tested | Kim et al., 2019 |
| Teratogenicity Study | | | | |
| SD Rats | 1250, 2500 and 5000 mg/kg bw | 10 days (days 6 to 15 of gestation) | No teratogenic effects observed at any dose of D-allulose | Annex G (Sa et al., 2020 - unpublished, supported by study report at Annex H (CCI)) |

#### *C.1.2.3.1 Reproductive and developmental study of Samyang's D-allulose (Kim et al., 2019)*

Kim et al. (2019) assessed the reproductive and developmental toxicity in rats of Samyang Corp.'s D-allulose derived from a non-genetically modified *M. foliorum*. The study was a one-generation assessment of reproductive outcomes in rats, in compliance with OECD Test Guideline 415. Prior to mating, F0 rats were dosed with 0, 500, 1,000, or 2,000 mg/kg D-allulose. The female rats were dosed continuously from 2 weeks before mating until day 21 of lactation while the males were dosed for 10 weeks before mating. No evidence of toxicity or mortality associated with D-allulose administration was observed. In addition, no statistically significant changes in body weight or eating behaviour were observed with D-allulose administration. Male body weights trended lower in the 1000 and 2000 mg/kg groups; however, these differences were not statistically significant.

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

In the F1 offspring of parents receiving D-allulose, the body weights were slightly higher on days 1 to 9 but after day 9 these effects were not evident. Physical development and behavioural functions were not altered in response to D-allulose administration of parent rats.

Given no adverse reproductive or developmental outcomes were observed, the study authors suggest the no-observed-adverse-effect level (NOAEL) of D-allulose was 2,000 mg/kg, the highest dose tested. The full study report supporting Kim et al. (2019) is provided at Annex M (CCI).

*C.1.2.3.2 Teratogenicity study of Samyang's D-allulose*

In a teratogenicity study with Samyang Corp.'s D-allulose (Sa et. al. 2020, unpublished – Annex G), Sprague Dawley (SD) rats were given repeated oral doses of 1,250, 2,500, or 5,000 mg/kg bw D-allulose on gestation days 6 to 15 (20 rats in each dose group). A solvent control group was also used. Rats in each group were evaluated for weight, animal death, corpus luteum number, implantation number, fetus weight, body length, tail length, live birth rate, fetal resorption rate, appearance malformation rate, visceral malformation rate and skeleton malformation rate.

No D-allulose related maternal toxicity, fetal malformation or death were observed in the study. No significant differences between the control group and dose groups were found in body weight, corpus luteum number, implantation number, live birth number, fetal resorption number, litter weight, body length, and tail length. No fetus appearance malformation or visceral malformation were observed. Sternum deficiency was reported in the 2,500 and 5,000 mg/kg bw D-allulose groups, as well as in the solvent control group. No significant differences in skeleton malformation rate were identified between the D-allulose dose groups and the solvent control group. No other skeleton malformations were observed in any group. The study concluded D-allulose did not exhibit teratogenic effects on pregnant SD rate under the test conditions. Details are presented in Annex G (unpublished manuscript) and supported by the study report at Annex H (CCI).

*C.1.2.3.3 Conclusion*

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

In addition, other sources of D-allulose had the oral $LD_{50}$ values in the range of 15.8-16.3 g/kg bw (Matsuo et al., 2002a). A 90-day sub-chronic toxicity study reported the NOAEL for D-allulose as 3% of the diet, the highest level tested (Matsuo et al., 2012). A 12- to 18- month

chronic toxicity study showed that D-allulose at the dose of 3% in the diet (or 1,280 mg/kg bw/day), the highest level tested, did not show adverse effects (Yagi and Matsuo, 2009).

### *C.1.2.4 Animal Efficacy Studies Reporting No Adverse Effects of D-Allulose*

As shown in Table C.1.2.4-1, several animal studies reported no adverse effects of D-allulose including Samyang (Kim et al., 2017) and other sources of D-allulose. These animal studies showed that D-allulose at the level of 5% in the diet (corresponding to up to 2,500 mg/kg bw/d) did not cause any adverse effects (Chung et al., 2012; Han et al., 2016; Hossain et al., 2012; Itoh et al., 2015; Kim et al., 2017; Matsuo and Izumori, 2004, 2006; Matsuo et al.2001a, 2001b, Nagata et al., 2015; Ochiai et al., 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. No studies reported adverse effects of D-allulose on the measured outcomes, such as body weight, glucose and lipid metabolism indicators, inflammatory biomarkers, serum or plasma adipocytokine concentrations, abdominal fat deposition and fecal microbiota.

As shown in Table C.1.2.4-1, administration of D-allulose resulted in various health benefits, such as reduced body weight gain with or without impacting food intake, reduced blood glucose and lipid concentrations, increased resting energy expenditure during darkness or changes in microbial community. Single dose studies or studies testing the effects of multi-components are not included in this review. Due to the substantial equivalence in purity and specifications, the findings from the studies are pertinent when evaluating the efficacy and safety of Samyang's D-allulose produced by *M. foliorum*.

Table C.1.2.4-1. Animal efficacy studies reporting no adverse effects of D-Allulose*

| Species | Dosage | Length | Primary endpoints | Results | Reference |
|---|---|---|---|---|---|
| Samyang's D-allulose | | | | | |
| 30 Male C57BL/6J *ob/ob* mice | 0 or 5% of diet | 12 weeks | Serum lipid profile (triacylglycerol, non-esterified fatty acids, total cholesterol, HDL-C, LDL-C); adipose tissue weight and adipose size | D-allulose vs. control: ↓ final body weight (50.99 vs. 55.77 g/d, P<0.05), adipose tissue (mesenteric fat: 2.60 vs. 3.06; perirenal fat: 6.16 vs. 7.01; total: 13.91 vs. 15.31% of body weight; P<0.05), adipocyte size (600,000 vs. 900,000 $\mu m^3$, P<0.001), and serum total cholesterol (293.41 vs. 376.93 mg/dL, P<0.05) | Kim et al., 2017 |
| Other Sources of D-allulose | | | | | |
| 36 Male C57BL/6J mice | 0 or 5% of diet | 16 weeks | Plasma lipid profile and adipokines (leptin, resistin, adiponectin); SCFA; fecal microbiota | D-allulose vs. high-fat diet: ↓ bw (32 vs. 42 g, P not specified); ↓ plasma leptin (5 vs. 19 ng/mL, P not specified) and resistin (140 vs. 220 ng/mL, P not specified); ↑ *Lactobacillus*, *Coprococcus*, and *Coprobacillus*; ↓ *Turicibacter*, *Clostridiaceae*, *Dorea*, and *Erysipelotrichaceae* | Han et al., 2020 |
| 16 Male Golden Syrian hamsters | 0 or 3% in normal diet | 8 weeks | Serum glucose, ALT, AST, insulin, proprotein convertase subtilisin/kexin type 9 (PCSK9), cholesterol, and TG; liver lipids, total cholesterol, TG, phospholipid, and glycogen; serum sterols | D-allulose vs. control: ↑ HDL-C levels (141 vs. 123 mg/dL, P<0.05); ↓ LDL/HDL ratio (0.231 vs. 0.282, P<0.05); ↓ serum PCSK9 (~32 vs. ~48 ng/mL, P<0.01). No significant differences in blood glucose, insulin, total cholesterol, AST and ALT levels. | Kanasaki et al., 2019 |
| 16 Male Golden Syrian hamsters | 0 or 3% in high fat diet | 4 weeks | | D-allulose vs. control: ↓ LDL-C levels (34.8 vs. 42.1 mg/dL, P<0.05); ↓ LDL/HDL ratio (0.219 vs. 0.261, P<0.05); ↓ serum PCSK9 (~23 vs. ~34 ng/mL, P<0.01). No significant differences in blood glucose, insulin, total cholesterol, AST and ALT levels. | |
| 60 Male C57BL/6J mice | 0 or 5% of high fat diet | 16 weeks | Plasma, hepatic, and fecal lipid profile (FFAs, phospholipid, apolipoprotein [Apo] AI, Apo B, HDL-C, TG, and total cholesterol); lipid-regulating enzyme activity; plasma leptin, resistin, and adiponectin | D-allulose vs. control: ↓ final body weight (30.13 vs. 39.38 g, P<0.05) and fat-pad mass; ↓ plasma leptin (1 vs. 19 ng/mL, P<0.05) and resistin (140 vs. 310 pg/mL, P<0.05); ↓ plasma lipids (free fatty acids: 0.41 vs. 0.52; total cholesterol: 2.99 vs. 4.17; triglyceride: 0.99 vs. 1.28; non-HDL-C: 1.84 vs. 3.00; LDL-C: 1.45 vs. 2.46 mmol/L; ApoB: 7.63 vs. 9.16 mg/dL; P<0.05); ↓ hepatic lipids (fatty acids: 0.13 vs. 0.175; triglyceride: 1.4 vs. 1.9; cholesterol: 0.24 vs. 0.3 mEq/g liver; P<0.05); ↑ fecal lipids (triglyceride: 29.5 vs. 15; cholesterol: 1,300 vs. 950; fatty acids: 460 vs. 350 nmol/g feces; P<0.05) | Han et al., 2016 |

| Species | Dosage | Length | Primary endpoints | Results | Reference |
|---|---|---|---|---|---|
| *ob/ob* and wild type C57BL/6J mice | 4 groups: 0, 2.5 or 5% of diet in *ob/ob* mice; 0% wild type mice control | 15 weeks | Body weight and body composition (body fat); hepatic steatosis using MRI; histological analysis of liver | 5% D-allulose vs. control in *ob/ob* mice: ↓ body weight (5% vs. 0%: 55 vs. 58 g, P<0.01) and liver weight (3.7 vs. 4.2 g, P<0.05); ↓ total fat mass (35 vs. 42 g, P<0.01) and abdominal visceral fat (5.4 vs. 6.2 g, P<0.01); improved hepatic steatosis | Itoh et al., 2015 |
| 45 Male diabetic (Otsuka Long-Evans Tokushima Fatty) rats | 5% of diet | 13 weeks | Obesity indicators; glucose metabolism (fasting blood glucose, oral glucose tolerance test, insulin resistance); inflammatory profile; serum adipocytokines (leptin and adiponectin) | D-allulose vs. control: ↓ body weight (500 vs. 600 g, P<0.001) and fat content (epididymal fat: 6 vs. 9.5; perirenal fat: 9 vs. 15 g/rat; P<0.01); ↓ AUC glucose (D-allulose vs. diabetic control ~23,000 vs. ~34,000, P<0.01); ↓ fasting blood glucose at week 13 (D-allulose vs. diabetic control vs. glucose vs. non-diabetic control:108 vs. 135 vs. 226 vs. 94 mg/dL); attenuated progressive pancreatic β-islet fibrosis and preserved pancreas islets | Hossain et al., 2012 |
| 31 Male Wistar rats | 5% of high sucrose diet | 8 weeks | Energy expenditure between week 5 and 7; body fat; liver weight, lipid and glycogen in liver and muscle; serum biochemistry (glucose, insulin, lipid profile, and adiponectin) | D-allulose vs. control: ↑ resting energy expenditure during darkness between 2 to 8 a.m. (~240 vs. ~200 KJ/6 h/kg, P<0.05); ↑ lipoprotein lipase activity in soleus muscle (4.8 vs. 3.8 U/g tissue, P<0.05); ↓ serum glucose (137.2 vs. 158.0 mg/100 mL, P<0.01), leptin (0.106 vs. 0.630 ng/mL, P<0.001), and adiponectin, glucose-6-phosphate dehydrogenase activities in liver (16.9 vs. 29.6 μ U/g tissue, P<0.01) and perirenal adipose tissue (0.16 vs. 0.66 μ U/g tissue, P<0.001), and body fat accumulation (28.8 vs. 41.9 g or 11.2 vs. 15.9% total body fat; P<0.001) | Ochiai et al., 2014 |
| 32 Male Wistar rats | 4 groups: 0 or 5% in high sucrose diet or high starch diet; rats previously fed high sucrose diet | 8 weeks | Body weights, dietary intakes; serum biochemistry (glucose, insulin, lipid profile, leptin, adiponectin, resistin, and obestatin; hepatic total lipids, TG, total cholesterol, and glycogen; carcass fat content, total body fat content | D-allulose vs. control: High sugar diet, ↓ bw gain (13 vs. 45 g, P<0.001), food efficiency (18.2 vs. 53.0 mg/g, P<0.001), intra-abdominal adipose tissue (23.85 vs. 32.38 g, P<0.001), and carcass fat (27.6 vs. 35.7 g, P<0.01);<br>High starch diet, ↓ body weight (22 vs. 37 g, P<0.05), food efficiency (25.1 vs. 40.9 mg/g, P<0.05), intra-abdominal adipose tissue (24.18 vs. 30.34 g, P<0.01), | Ochiai et al., 2013 |

| Species | Dosage | Length | Primary endpoints | Results | Reference |
|---|---|---|---|---|---|
| 50 Male Sprague-Dawley rats | Previous fed high fat diet for 4 wk and switched to 0, 2.5, or 5% D-allulose, 5% sucrose, or 5% erythritol in normal diet | 52 days | Feed intake, bw and food efficiency ratio; tissue weight; serum biochemistry (TG, HDL-C) | 5% D-allulose vs. 0% normal diet: ↓ weight gain (1.7 vs. 2.4; 5%: 1.3 vs. 2.4; P<0.05) and food efficiency ratio (0.06 vs. 0.12, P<0.05); ↓ epididymal (10.19 vs. 16.35, P<0.05), perirenal adipose tissue weight (5%: 3.13 vs. 5.30; P<0.05), and retroperitoneal adipose tissue weight (11.10 vs. 17.93 g, P<0.05); ↑ liver weight (18.18 vs. 13.44 g, P<0.05); no difference in serum cholesterol/HDL-C and LDL-C/HDL-C ratios | Chung et al., 2012 |
| 40 male Sprague-Dawley rats | 0 or 5% D-allulose, 5% sucrose, or 5% erythritol in high fat diet to rats previously fed high fat diet for 4 wk | | | 5% D-allulose vs. control high fat diet: ↓ weight gain (1.9 vs. 3.8 g/d, P<0.05), food efficiency ratio (0.09 vs. 0.15; P<0.05); ↓ epididymal (17.36 vs. 21.54 g; P<0.05), and perirenal adipose tissue weight (4.97 vs. 6.81 g; P<0.05; no difference in serum cholesterol/HDL-C and LDL-C/HDL-C ratios; no difference in serum cholesterol/HDL-C and LDL-C/HDL-C ratios | |
| 40 Male C57BL/6J *db*/*db* mice and 10 wild-type mice | 0.2 g/kg bw/day | 28 days | Plasma glucose and insulin, and oral glucose tolerance test; lipid profile in plasma, liver, and feces (TG, total cholesterol, HDL-C, and LDL-C) | D-allulose vs. control in diabetic rats: ↓ weight gain (4.75 vs. 7.50 g/day, P<0.05); ↓ postprandial blood glucose concentration (at week 4; ~16 vs. ~23 mM, P<0.05); improved glucose tolerance and AUC for glucose; no effect on serum insulin; ↑ LDL-C/HDL-C ratio (1.67 vs. 0.90, P<0.05); ↓ hepatic TG (164.36 vs. 264.58 mg/g, P<0.05) and total cholesterol (64.53 vs. 173.91 mg/g, P<0.05) | Baek et al., 2010 |
| 48 Sprague Dawley rats | 3% of diet | 4 weeks | Serum and tissue biochemistry (serum glucose, lipid, insulin, leptin, cholesterol, phospholipid, hepatic TG); liver enzyme activity | D-allulose vs. control: ↓ serum insulin (time 0900: 1.2 vs. 2.2; 0300: 1.3 vs. 3 ng/dL; P<0.05) and leptin (time 1500: 10 vs. 14 ng/dL, P<0.05); ↓ glucose-6-phosphate dehydrogenase (25 vs. 43 nmol/min/mg protein, P<0.05) and malic enzyme (29.9 vs. 47.5 nmol/min/mg protein, P<0.05) | Nagata et al., 2015 |
| 16 Male Sprague Dawley rats | | | Energy expenditure | D-allulose vs. control: ↓ body weight (389 vs. 426 g, P<0.05) and food intake (23.8 vs. 25.7 g/d, P<0.05); ↑ energy expenditure in light period ( 6.0-8.0 vs. 5.5-7.0 kcal/kg bw/h, P<0.05) and fat oxidation in dark period (0.3-0.35 vs. 0.22-0.24 g/kg bw/h, P<0.05) | |

| Species | Dosage | Length | Primary endpoints | Results | Reference |
|---|---|---|---|---|---|
| 18 Male Wistar rats | 5% in the diet | 3 weeks | Plasma glucose, insulin, and TG; enzyme activities in various tissues | D-allulose vs. D-fructose: ↓ abdominal adipose tissue weight (6.8 vs. 10.5 g, P<0.05); ↓ liver fatty acid synthase (57.4 vs. 91.0 U/g tissue, P<0.05) and glucose-6-phosphate dehydrogenase (872 vs. 1,568 mU/g tissue, P<0.05); no difference in lipoprotein lipase activities in heart, soleus muscle, perirenal adipose tissue, and subcutaneous adipose tissue | Matsuo et al., 2001a |
| 24 Male Wistar rats | 5% in the diet | 28 days | Plasma glucose, TG, total cholesterol, and insulin; enzyme activity in various tissues | D-allulose vs. glucose: ↓ abdominal adipose tissue weight (6.8 vs. 9.6 g, P<0.05); ↓ liver fatty acid synthase (66.7 vs. 80.2 U/g tissue, P<0.05); D-allulose vs. fructose: ↓ abdominal adipose tissue weight (6.8 vs. 9.8 g, P<0.05); ↓ liver fatty acid synthase (66.7 vs. 87.2 U/g tissue, P<0.05) and glucose-6-phosphate tissue (1673 vs. 2211 mU/g tissue, P<0.05) | Matsuo et al., 2001b |
| 24 Male Wistar rats | 0 or 5% in high (25% fat) or low fat (5% fat) diets | 16 weeks | Oral glucose tolerance test; bw, tissue weights, and carcass composition; liver glycogen protein and TG; serum conc. of glucose, insulin, TG, free fatty acids, and adipocytokines | No effect on body fat accumulation, glucose tolerance, serum concentrations of glucose, insulin, TG, free fatty acids, leptin, adiponectin, and TNF-α. D-allulose diet increased liver weight and protein content. | Matsuo and Izumori, 2004 |
| 48 Male Wistar rats | 5% in the diet | 8 weeks | Diurnal rhythm of plasma glucose and insulin levels; serum glucose and tissue glycogen; carcass composition; serum glycoalbumin | D-allulose vs. control: ↓ plasma glucose (increment of AUC: 2,465 vs. 2,617 h·mg/100 mL, P<0.05); ↑ plasma insulin (increment of AUC: 125 vs. 116 h·ng/mL, P<0.05); ↓ weight gain (99 vs. 113 g. P<0.05); ↑ liver glycogen before (70 vs. 43 mg/tissue, P<0.05) and after meals (99 vs. 55 mg/tissue, P<0.05) | Matsuo and Izumori, 2006 |

*Studies with single dose or with mixture of various components are not included in this review. Apo = apolipoprotein; AU C= area under the curve; bw = body weight; d = day; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; TG = triglycerides; TNF-α = tumor necrosis factor α.

*C.1.2.4.1 An efficacy study of Samyang's D-allulose*

In a study by Kim et al. (2017), C57BL/6J ob/ob mice were fed with a control or 5% D-allulose diet for 12 weeks. D-Allulose decreased final body weight, adipose tissue mass, and adipocyte size partly due to calorie dilution effects. Additionally, serum concentrations of total cholesterol were reduced in the test group. No adverse effects of Samyang's D-allulose were reported on the measured outcomes.

*C.1.2.4.2 Efficacy studies of other sources of D-allulose*

*C.1.2.4.2.1 A study by Han et al. (2020)*

In a study by Han et al. (2020), 36 C57BL/6J mice were divided into 4 dietary groups and fed a normal diet, a high-fat diet (20% fat, 1% cholesterol, w/w), and a high-fat diet with 5% erythritol and D-allulose supplement for 16 weeks. A pair-feeding approach was used so that all groups receiving the high-fat diet would have the same calorie intake. As a result, body weight and body fat mass in the D-allulose group were significantly decreased toward the level of the normal group with a simultaneous decrease in plasma leptin and resistin. Fecal SCFA production analysis revealed that D-allulose induced elevated total SCFA production compared to the other groups. Also, D-allulose supplement induced the change in the microbial community that could be responsible for improving the obesity based on 16S ribosomal RNA (rRNA) gene sequence analysis, and D-allulose significantly increased the energy expenditure on Day (6 am to 6 pm). Taken together, the findings suggest that 5% dietary D-allulose led to an improvement in high-fat diet-induced obesity by altering the microbiome community.

*C.1.2.4.2.2 A study by Kanasaki et al. (2019)*

Kanasaki et al. (2019) studied the effects of D-allulose on the cholesterol metabolism in Golden Syrian hamsters. Hamsters received one of four diets for 4 or 8 weeks: normal diet with or without 3% D-allulose (8 weeks), or high-fat diet with or without 3% D-allulose (4 weeks). No significant differences were noted in body weight, abdominal fat weight, and serum concentrations of glucose, insulin, TG, and total cholesterol between the groups. However, D-allulose significantly increased fasting blood concentrations of HDL-C in normal diet-fed hamsters and decreased LDL-C levels in high-fat diet-fed hamsters, causing a decrease in the LDL/HDL ratio. The D-allulose group had higher liver TG concentration in normal diet-fed rats only, but not in high-fat diet-fed rats. Dietary D-allulose decreased serum proprotein convertase subtilisin/kexin type 9 levels in both diets. The authors concluded that D-allulose may favorably modulate cholesterol metabolism by reducing proprotein convertase subtilisin/kexin type 9 in hamsters.

*C.1.2.4.2.3 A study by Han et al. (2016)*

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 weeks. 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.

*C.1.2.4.2.4 A study by Itoh et al. (2015)*

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 sub-chronic 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 by the 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 sub-chronic 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.

*C.1.2.4.2.5 A study by Hossain et al. (2012)*

A study by Hossaine et al. (2012) examined the effect of D-allulose on the protection of pancreatic β-islets using Otsuka Long-Evans Tokushima Fatty rats, a type 2 diabetes mellitus model. These diabetic rats were fed with 5% D-allulose or 5% D-glucose supplemented drinking water for 13 weeks. Water only was given to diabetic and non-diabetic Long-Evans Tokushima Otsuka rats which served as diabetic and non-diabetic controls. D-allulose significantly reduced the increase in body weight (109.3 vs. 114.8 g) and abdominal fat deposition (13.2 vs. 25.4 g). D-allulose group had significantly lower fasting periodical blood concentration and attenuated glycemic responses in the OGTT, which was conducted before sacrifice at week 13 (D-allulose vs. diabetic control vs. glucose vs. non-diabetic control: fasting blood glucose, 108 vs. 135 vs. 226 vs. 94 mg/dL, $P<0.05$ for diabetic control and glucose; $AUC_{glucose}$, ~23,000 vs. ~ 34,000 vs. ~30,000 vs. ~16,000, $P<0.01$ for diabetic control). In addition, D-allulose significantly attenuated progressive pancreatic β-islet fibrosis and preserved islets, as evaluated by hematoxylin–eosin staining, Masson's trichrome staining, and immunostainings of insulin and α-smooth muscle actin. Serum concentrations of leptin was significantly lower in the D-allulose group than in the control group at the end of the experiment period (D-allulose vs. diabetic control, 15 vs. 22 ng/mL, $P<0.01$). The authors concluded that D-allulose protected and preserved pancreatic β-islets through the maintenance of hyperglycemia and by the prevention of fat accumulation in Otsuka Long-Evans Tokushima Fatty rats.

*C.1.2.4.2.6 Studies by Ochiai et al. (2013, 2014)*

Ochiai et al. (2014) investigated the effects of D-allulose on energy expenditure, body fat, and serum glucose and lipid profiles in rats to find out if D-allulose has an anti-obesity effect. Wistar rats were divided into three dietary groups: pair-fed high-sucrose diet containing 5% D-allulose or 5% cellulose (control), and control diet ad libitum. Most of the comparisons discussed in this summary will focus on the pair-fed control. D-allulose intake significantly increased resting energy expenditure during darkness and lipoprotein lipase activity in the soleus muscle compared to the pair-fed control while increasing serum levels of glucose, leptin, and adiponectin, glucose-6-phosphate dehydrogenase activities in the liver, perirenal adipose tissue, and body fat accumulation. The data suggest that anti-obesity effects of D-allulose could be induced by suppressing lipogenic enzyme activity and by increasing energy expenditure in rats.

Ochiai et al. (2013) evaluated the anti-obesity effects of dietary D-allulose in adult rats fed a high-sucrose diet. Wistar rats (16 weeks old) that had previously been fed a high-sucrose diet were fed a high-sucrose diet or a high-starch diet with or without 5% D-allulose for 8 weeks. D-allulose decreased food efficiency, carcass fat percentage, abdominal fat accumulation, and body weight gain.

*C.1.2.4.2.7 A study by Chung et al. (2012)*

To study the anti-obesity effects of D-allulose, Chung et al. (2012) fed either a normal diet or high fat diet to Sprague–Dawley rats for 8 weeks after inducing obesity by feeding a high fat diet for 4 weeks. In experiment 1, 50 obese rats were switched to one of 5 normal diets (0, 2.5 or 5% D-allulose, 4% sucrose, or 5% erythritol) for 8 weeks. In experiment 2, 40 obese rats were assigned to one of 4 high fat diets (supplemented with 0% or 5% D-allulose, 5% sucrose, or 5% erythritol) for 8 weeks. D-allulose groups exhibited lower weight gain, food efficiency ratio, and fat accumulation than erythritol or sucrose-fed rats. This anti-obesity effect of D-allulose was more prominent in normal diet than with high-fat diet. The data suggest that the combination of D-allulose and fat restriction have a synergistic effect in reducing obesity. D-allulose inhibited the differentiation of mesenchymal stem cell to adipose tissue in a dose-dependent manner; the effect was greater than that of erythritol. D-allulose increased liver weight with normal diet than with the high fat diet. No differences were noted in serum cholesterol/HDL-C and LDL-C/HDL-C ratios However, no treatment-related abnormalities were observed from histopathological examination of the liver. The authors concluded that D-allulose could reduce obesity.

*C.1.2.4.2.8 A study by Nagata et al. (2015)*

In a study by Nagata et al. (2015), the 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, 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.

*C.1.2.4.2.9 A study by Baek et al. (2010)*

In the study by Baek et al. (2010), the effects of D-allulose on glycemic responses, insulin release, and lipid profiles were compared with those of D-glucose and D-fructose in C57BL/6J *db/db* mice, a genetic diabetes model. Diabetic rats were subjected to one of 4 treatments for 4 weeks: 200 mg/kg body weight of D-allulose, D-glucose, D-fructose, or water (control). In addition, wild type mice were supplemented with water to serve as a non-diabetic control. Rats fed D-allulose had significantly lower weight gain compared to other diabetic rats (non-diabetic control vs. D-allulose vs. diabetic control vs. D-glucose vs. D-fructose: 2.75 vs. 4.75 vs. 7.5 vs. 7.87 vs. 7.63 g/day, $P<0.05$). Diabetic mice consuming D-glucose and D-fructose or water had more than two-fold increase in postprandial blood glucose level. However, D-allulose attenuated the increase in blood glucose levels (Week 4: non-diabetic control vs. diabetic control vs. other

2 sugar groups: D-allulose; 7 vs. 23 vs. 17 vs. 22 mM, P < 0.05). D-allulose significantly improved glucose tolerance and the areas under the curve for glucose (P < 0.05) but had no effect on serum insulin concentration and the plasma lipid profile. Hepatic TG and cholesterol concentrations were increased by 66 to 288%, respectively, in diabetic rats compared to non-diabetic control rats. The administration of D-allulose attenuated increases in hepatic concentrations of TG and total cholesterol by 37.9% and 62.9%, respectively, compared to the diabetic control (P < 0.05). No adverse effects were noted.

*C.1.2.4.2.10 A study by Matsuo et al. (2001a)*

Matsuo et al. (2001a) studied the effects on body fat accumulation of D-allulose compared with cellulose or D-fructose in rats. Wistar male rats were fed experimental diets including 5% D-allulose, cellulose or D-fructose for 21 days. Abdominal adipose tissue weight was lower (P <0.05) in rats fed D-allulose than in those fed D-fructose. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were lower (P<0.05) in rats fed D-allulose, whereas lipoprotein lipase activities in the heart, soleus muscle, perirenal adipose tissue, and subcutaneous adipose tissue did not differ. These results suggest that supplementation of D-allulose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed D-allulose might have resulted from lower lipogenesis in the liver. No adverse effects were reported. The authors concluded that D-allulose could prove to be a good sugar substitute.

*C.1.2.4.2.11 A study by Matsuo et al. (2001b).*

Wistar male rats were fed experimental diets that consisted of 5% D-allulose, cellulose, D-fructose, or D-glucose for 28 days (Matsuo et al., 2001b). Abdominal adipose tissue weight was lower (P < 0.05) in rats fed the D-allulose diet than in rats fed D-fructose and D-glucose diets, even though the four dietary groups were offered the same amount throughout the experimental period. Fatty acid synthase and glucose 6-phosphate dehydrogenase activities in the liver were lower (P < 0.05) in rats fed the D-allulose diet than in rats fed the D-fructose and D-glucose diets. However, lipoprotein lipase activities in the heart, soleus muscle, and perirenal adipose tissue were the same. These results suggest that a supplement of D-allulose in the diet suppresses hepatic lipogenic enzyme activities. The lower abdominal fat accumulation in rats fed the D-allulose diet might result from lower lipogenesis in the liver. No adverse effects were reported.

*C.1.2.4.2.12 A study by Matsuo and Izumori (2004)*

Effects of supplemental D-allulose on glucose tolerance and secretion of adipocytokines were evaluated in 24 male Wistar rats (4 weeks old) fed a high-fat and low-fat diets **(**Matsuo and Izumori, 2004**)**. Rats received one of the following 4 diets: a high-fat (25% fat) or a low-fat (5% fat) diet containing 5% D-allulose or cellulose, for 16 weeks. Abdominal adipose tissue weights and carcass fat content were greater in rats fed the high-fat diet. Glucose tolerance decreased with time. However, no significant differences were observed in body fat accumulation, glucose tolerance, serum concentrations of glucose, insulin, TG, free fatty acids, leptin, adiponectin, and tumor necrosis factor-α among the groups. D-allulose diet increased liver weight and protein content. In the discussion part, the authors presented that D-tagatose supplementation also increased liver weight and protein content in rats (Bar, 1999). It was hypothesised that ketohexoses, such as D-allulose and D-tagatose, stimulate the glycogen synthesis from glucose in the liver by controlling the enzymes that control glycogen synthesis and phosphorylation of

glucose to glucose-6-phosphate by glucokinase, which is known as a rate-limiting step in glucose utilisation in the liver.

*C.1.2.4.2.13 A study by Matsuo and Izumori (2006)*

Matsuo and Izumori (2006) studied the effects of supplemental D-allulose in the diet on diurnal variation in plasma glucose and insulin concentrations in rats. A total of 48 male Wistar rats were divided into four groups. Each group, except for the control group, was fed a diet of 5% D-fructose, D-allulose, or a 3:1 mixture of D-fructose and D-allulose for 8 weeks. The D-allulose group had lower body weight gain and plasma glucose concentrations, but higher plasma insulin concentrations and liver glycogen content than the control and fructose groups.

*C.1.2.4.2.14 Summary*

Several mechanisms of actions have been proposed to explain the potential mechanisms of anti-obese and anti-hyperglycemic effects of D-allulose:

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 (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);
6) enhanced insulin sensitivity (Hossain et al., 2012) and/or
7) altered hepatic glucose metabolism via the translocation of glucokinase (Hossain et al., 2012).

As shown in Table C.1.2.4-1, 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.

### *C.1.2.5 Mutagenicity and genotoxicity studies*

The identified mutagenicity and genotoxicity studies conducted on D-allulose are summarised in Table C.1.2.5-1. In all the identified studies, D-allulose was reported not to produce any mutagenic or genotoxic effects.

Table C.1.2.5-1 Summary of *in vitro* mutagenicity/genotoxicity studies of D-allulose

| Test | Concentration | Reference |
|---|---|---|
| Samyang's D-allulose | | |
| *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537 and *Escherichia coli* WP2urvA | 0, 61.7, 185, 556, 1670, or 5000 µg/plate | Kim, 2018 |
| Mammalian chromosome aberration test using Chinese hamster ovarian fibroblast cells | 1.25, 0.08, or 5 mg/mL | Kim, 2018 |
| Micronucleus test using ICR mice | 500, 1000, or 2000 mg/kg | Kim, 2018 |
| Other sources of D-allulose | | |
| Four histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and a tryptophan-dependent strain of *Escherichia coli* (WP2 urvA(pKM101)) | 5,000 ug/mL | GRN 400 |
| Micronucleus test using CD1 mice | 2,000 mg/kg/d | GRN 400 |
| Chromosomal aberration test | 1,800 ug/mL | GRN 400 |

#### *C.1.2.5.1 Mutagenicity and genotoxicity studies of Samyang's D-allulose*

Mutagenicity and genotoxicity studies of Samyang's D-allulose reported that it was not mutagenic or genotoxic (Table C.1.2.5-1)). Studies of other sources of D-allulose also found that D-allulose was not mutagenic or genotoxic. In addition, *M. foliorum* SYG27B-MF, Samyang's production organism for D-allulose, was not mutagenic and genotoxic (more detail on the safety of *M. foliorum* SYG27B-MF is provided in section C.2).

##### *C.1.2.5.1.1 Mutagenicity study of Samyang's D-allulose (Kim, 2018)*

In order to detect the mutagenicity of the test substance, a reverse mutation test was conducted with histidine auxotrophic mutants, such as *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and a tryptophan auxotrophic mutant, such as *Escherichia coli* WP2uvrA. The test substance was prepared by dissolving it in sterile distilled water. For the concentration determination test, 5 different concentrations (0, 312, 625, 1250, 2500, and 5000 ㎍/plate) were set, with 5000 ㎍/plate as the maximum concentration and other concentrations following a division factor of 2. Sterile distilled water served as the negative control. Sodium azide ($NaN_3$; -S9), 9-aminoacridine (9-AA; -S9), 4-nitroquinoline-N-oxide (4-NQO; -S9) and 2-aminoanthracene (2-AA; +S9) were used as the positive controls.

The result of the concentration determination test showed no increase in the number of reverse mutation colonies, and no overt cytotoxicity was observed for any of the strains regarding all concentrations. Therefore, following the OECD Guidelines, 5 different concentrations were used for this test (0, 61.7, 185, 556, 1670, and 5000 ㎍/plate) with 5000 ㎍/plate as the maximum concentration and other concentrations following a division factor of 3. In a test conducted with all 5 strains to determine whether the test conditions were appropriate, the frequency of colony formation by reverse mutation in the positive control group was 2~10 times significantly higher

than the solvent control group regardless of the presence of the metabolic activator; thus, the test conditions and conclusion were appropriate.

The result of the mutagenicity test in all five strains (TA98, TA100, TA1535, TA1537, and WP2uvrA) showed no reproducible increase in the number of colonies, which is seen as a positive factor for reverse mutation induction. Regardless of the presence of the metabolic activator, no dose-response relationship was observed. These results demonstrated that the test substance, D-allulose, did not cause reverse mutation in the 5 strains and is, thus, negative. The full study report for this reverse mutation test is available at Annex N (CCI).

*C.1.2.5.1.2 Genotoxicity study of Samyang's D-allulose using Chinese hamster ovarian fibroblast (CHO-K1) cells (Kim, 2018)*

The purpose of this study was to evaluate the genotoxicity of D-allulose using Chinese hamster ovarian fibroblast (CHO-K1) cells. The test substance was prepared by dissolving it in sterile distilled water.

Results of the cell proliferation inhibition test showed that the inhibition of cell proliferation by more than 50% was not seen in any of the concentrations, including the highest concentration of 5 mg/mL, in the presence or absence of the metabolic activator (S9 mix) for 6 hours and in the absence of the S9 mix for 24 hours. Therefore, for this experiment, all three groups followed a three-step concentration administration plan of 0.08 mg/mL, 1.25 mg/mL, and 5 mg/mL, the highest dose concentration.

The 6-hour groups with and without S9 mix did not show significant differences in numerical or structural chromosomal anomalies compared to the negative control group at the 3 concentration levels ($p>0.01$). The 24-hour metabolic activator-absent group did not show significant differences in numerical or structural chromosomal anomalies compared to the negative control group at the 3 concentration levels either ($p>0.01$). All the groups showed significant differences in numerical and structural chromosomal anomalies when treated with the positive control material and compared to the negative control group. Thus, the conditions in this study are considered appropriate.

These results demonstrated that the test substance, D-allulose, does not cause numerical or structural chromosomal anomalies under these conditions. The full study report for this genotoxicity study is available at Annex O (CCI).

*C.1.2.5.1.3 In vivo mouse micronucleus test of Samyang's D-allulose (Kim, 2018)*

The genotoxic potential of D-allulose was determined in a micronucleus test using bone marrow cells in ICR mice. Mice (5 animal per group) were administered 500, 1000, or 2000 mg/kg D-allulose, a negative control (sterile distilled water), or a positive control (mitomycin C) for 2 days to observe the frequency of micronucleus formation for up to 48 hours. The comparison between the proportions of polychromatic erythrocytes in total erythrocytes showed no significant difference between the negative control group and the test groups. Also, when comparing the average frequency of micronucleated polychromatic erythrocytes, there was no significant difference between the negative control group and the test groups. No dose-response relationship was observed. The results demonstrated that D-allulose did not cause micronucleus formation in the mouse bone marrow cells under these conditions. The average frequency of micronucleus formation in polychromatic erythrocytes was significantly higher in the positive control group compared to the negative control group; thus, the test conditions were

appropriate for examining the frequency of micronucleus formation in the test substance. The full study report for this *in vivo* mouse micronucleus test is available at Annex P (CCI).

*C.1.2.5.2 Mutagenicity and genotoxicity studies of other sources of D-allulose*

Results from an Ames test, a micronucleus test, and a chromosomal aberration test of another source of D-allulose were reported in GRN 400 from manufacturer, CJ CheilJedang. These results are reproduced below (GRN 400).

*C.1.2.5.2.1 Ames test:*
Four histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and a tryptophan-dependent strain of *Escherichia coli* [WP2 urvA(pKM101)] were used to evaluate the mutagenic potential of D-allulose (up to 5,000 ug/plate) (GRN 400). No mutagenic potential of D-allulose was observed.

*C.1.2.5.2.2 Micronucleus test:*
In the micronucleus test using CD1 mice, no significant increase was observed in micronucleated polychromatic erythrocytes (MPCEs) at any concentration up to 2,000 mg/kg/d of D-allulose compared with vehicle control (GRN 400).

*C.1.2.5.2.3 Chromosomal aberration test:*
D-allulose at a dosage of 1,800 µg/mL did not induce an increase in the number of chromosomal aberrations (GRN 400).

*C.1.2.5.3 Summary of Mutagenicity and Genotoxicity Studies*

Overall, the data from studies indicate that Samyang's D-allulose and other sources of D-allulose were not mutagenic or genotoxic.

*C.1.2.6 Human clinical studies*

The safety of D-allulose is supported by an extensive number of clinical studies, many of which address the primary safety issues regarding D-allulose. Table C.1.2.6.1-1 lists the studies summarised below in relation to gastrointestinal tolerance studies (section C.1.2.6.1). Table C.1.2.6.1-2 lists the studies summarised below relating to other effects studied in human clinical trials (sections C.1.2.6.2 to C.1.2.6.6).

*C.1.2.6.1 Tolerance studies*

*C.1.2.6.1.1 A study by Iida et al. (2007)*

Iida et al. (2007) reported that D-allulose intakes up to 0.6 g/kg bw was well tolerated in 5 healthy males and 5 healthy female subjects aged 20-30 years. The subjects ingested D-allulose at the level of 0.4 g/kg bw for the first dose. Those with severe diarrhea were excluded from the test group for the next test procedure. For those without diarrhea, the dosage of D-allulose was increased by 0.1 g each time up to 0.9 g/kg bw with a week of wash-out period between treatments. No diarrhea was reported when the subjects consumed D-allulose at levels of 0.4-0.5 g/kg bw When the dosage was escalated to 0.6 g/kg bw, diarrhea occurred in 1 male. At the level of 0.7 g/kg bw, 2 females experienced diarrhea. Two males and 3 females had diarrhea at the dosage of 0.8 g/kg bw. With the dosage of 0.9 g/kg bw, 2 males did not suffer from diarrhea. The authors concluded that the maximum non-effect level of D-allulose in

causing diarrhea was estimated as 0.5 g/kg bw for males, 0.6 g/kg bw for females, and 0.55 g/kg of bw for mean value. These levels may correspond to 30-36 g for an adult weighing 60 kg.

*C.1.2.6.1.2 A study by Han et al. (2018b)*

Han et al. (2018b) conducted a gastrointestinal tolerance test of D-allulose in healthy young adults in a non-randomised, controlled trial order to establish its daily acceptable intake level. When the dose of D-allulose was gradually increased in steps of 0.1 g/kg bw to identify the maximum single dose for occasional ingestion, no cases of severe diarrhea or gastrointestinal symptoms were noted until a dose of 0.4 g/kg bw was reached. Severe symptoms of diarrhea were noted at a dose of 0.5 g/kg bw. Similarly, the gastrointestinal tolerance test did not show any incidences of severe diarrhea or gastrointestinal symptoms, such as diarrhea and abdominal pain, until a dose of 0.5 g/kg bw was reached.

Increasing the total daily D-allulose intake gradually to 1.0 g/kg bw for regular ingestion resulted in incidences of severe nausea, abdominal pain, headache, anorexia, and diarrheal symptoms. Based on these results, a maximum single dose and maximum total daily intake of D-allulose were determined to be 0.4 g/kg bw/single dose and 0.9 g/kg bw/day, respectively. These maximum tolerable levels of D-allulose are similar to that of erythritol (0.66 g/kg bw/day or 45 g per person per day). 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 (Institute of Medicine [IOM], 2005).

Overall, studies found that daily doses up to 0.9 g/mg kg bw were well tolerated. This level may correspond to 54 g/person/day for an adult weighing 60 kg. The gastrointestinal tolerance level of D-allulose is comparable to those of D-erythritol and D-tagatose.

*C.1.2.6.1.3 Gastrointestinal tolerance and safety of D-tagatose*

Donner et al. (2010) reported that consumption of D-tagatose, at a daily dose of 45 g (15 g three times a day) for 1 year, did not induce any adverse effects, such as gastrointestinal tolerance and blood uric acid concentration, in 8 subjects with type 2 diabetes mellitus. No serious adverse effects were seen during the 12-month treatment period although ten of the initially 12 recruited subjects experienced gastrointestinal side effects that were mild and transient. Saunders et al. (1999) reported that daily doses of 75 g (25 g, 3 times a day) were well tolerated. A transient increase in blood uric acid concentrations appeared after a single dose of 75 g of D-tagatose in the tolerance test. However, a repeated daily dose of 75 g for 8 weeks had no impact on fasting plasma concentrations of magnesium, phosphorus, cholesterol, TG, $HbA_{1c}$, glucose, and insulin. Overall, daily doses up to 75 g were well tolerated with no side effects.

*C.1.2.6.2 Effects of D-allulose on glucose metabolism in humans*

C.1.2.6.2.1 A 48- to 52-week human clinical study with D-allulose

In a 48-week feeding study by Tanaka et al. (2020), 90 subjects were randomly assigned to one of 3 daily test beverages containing 0 (control), 5 (low-dose), and 15 g (high-dose) D-allulose for 48 weeks, with a follow up after 4 weeks at week 52. Eight subjects were lost to follow-up and 82 completed the study. Clinical examinations were performed every eight weeks, beginning

from initial consumption until week 52. Long term administration of D-allulose resulted in no significant changes in lipid metabolism indicators and inflammatory biomarkers.

A 75 g OGTT was conducted on the first day of the consumption and 48 weeks after starting consumption. On examination days, the subjects took the test beverage after finishing the examination. No significant changes in glucose area under the curve (ΔAUC) on OGTT were found in all subjects because half of them were within normal range for glucose metabolism. However, in the sub-group with borderline diabetes, the differential values of glucose ΔAUC between baseline and week 48 were significantly decreased in the high D-allulose group than in the placebo (high-dose vs. low-dose vs. control: -4.7 vs. 8.3 vs. 18.1 mg*hr/dL, P<0.01 for high-dose). It is noteworthy that the placebo borderline diabetes sub-group group had a significant increase in glucose ΔAUC at week 48 when only compared with week 0 (76.4 vs. 58.3 mg*hr/dL; P<0.05). In the sub-group with the borderline diabetes, HbA1c values in the D-allulose groups did not vary. On the other hand, a placebo group had a significant increase at week 40 (6.13 vs. 5.91%, P<0.05).

Overall, the authors concluded that long-term D-allulose consumption significantly improved glucose metabolism as measured by ΔAUC on OGTT.

*C.1.2.6.2.2 12-week human clinical studies of D-allulose*

A study by Han et al. (2018b) reported no adverse effects of D-allulose (source, CJ CheilJedang, Korea) on blood glucose metabolism indicators and inflammatory biomarkers in 144 healthy overweight or obese subjects. Subjects were randomised to receive either 0, 8, or 14 g D-allulose for 12 weeks in a placebo-controlled randomised controlled trial. Twenty-three were lost to follow-up so 121 subjects were analysed. No significant differences were observed in fasting blood glucose, $HbA_{1c}$, insulin, HOMA-IR, ghrelin, gastric inhibitory polypeptide, and/or plasminogen activator inhibitor-1 among the groups (Han et al., 2018b).

In a 12-week randomised double-blind, placebo-controlled parallel study by Hayashi et al. (2010), 17 normal subjects consumed 5 g of D-allulose or D-glucose with meals three times a day (a total of 15 g/day) for 12 weeks. No treatment-related abnormalities were observed in glucose metabolism indicators such as fasting blood levels of glucose, insulin, and $HbA_{1c}$.

In each of these studies, no adverse effects were reported on fasting blood concentrations of glucose and insulin as well as glycosylated hemoglobin values.

*C.1.2.6.2.3 Short-term human clinical studies*

Iida et al. (2008) evaluated the ability of D-allulose to suppress the elevation of blood glucose and insulin concentration in a dose-dependent manner in a single-blind, randomised crossover study. Twenty young volunteers (aged 20-39 years) were randomised to receive one of five single dose test beverages: 7.5 g D-allulose alone, 75 g maltodextrin alone, 75 g maltodextrin + 2.5 g D-allulose, 75 g maltodextrin + 5 g D-allulose, or 75 g maltodextrin + 7.5 g D-allulose. Blood was collected before intake and over the 120-minute period after intake. Allulose consumption at a single dose of 5 or 7.5 g significantly reduced plasma glucose AUC associated with the load test with 75 g maltodextrin in a dose response manner (control, 2,138; 2.5 g, 1,847; 5 g, 1,533; 7.5 g, 1,468 uU.minute/mL; P<0.01 for 5 to 7.5 g compared to the control). Independent administration of 7.5 g D-allulose without maltodextrin had no influence on blood glucose or insulin concentration. D-allulose is considered efficacious in the suppression of elevated blood glucose concentration after eating in humans.

Hayashi et al. (2010) investigated the effect of a single dose of 5 g D-allulose on postprandial blood glucose levels in 30 adult men and women, including borderline diabetes patients in a randomised, double-blind, placebo-controlled crossover experiment. Four subjects dropped out, thus, 26 completed the study. The subjects consumed 0 or 5 g of D-allulose in tea with a standard meal, and glycemic responses were monitored over a 120 minute period. Blood glucose decreased in the D-allulose group compared to placebo at 30 minutes (161.6 vs. 174.0 mg/dl; $P<0.05$) and 60 minutes (173.6 vs. 180.5 mg/dl; $P<0.05$) after consumption of the test meal. Similarly, the AUC for the test meal was significantly decreased in the D-allulose group (5,739 vs. 6,482 mg*min/dl, $P<0.01$). Further, blood insulin level also decreased compared to control (42.1 vs. 48.5 uU/ml, $P<0.05$) at 30 minutes. The results suggest that D-allulose was effective in suppressing the postprandial blood glucose elevation mainly in borderline diabetes cases.

Noronha et al. (2018) compared the effect of small doses of fructose and allulose on postprandial blood glucose regulation in 24 subjects with type 2 diabetes in a crossover design with more than 1-week washouts. Treatments consisted of fructose or allulose at 0 (control), 5, or 10 g added to a 75-g glucose solution. A standard 75 g OGTT protocol was followed with blood sample collections over the 120-minute post administration period. Allulose significantly reduced plasma glucose incremental AUC in a dose response manner: 10 g compared with 0 g had an 8% reduction in incremental AUC, although these reductions were within the pre-specified equivalence margins of ±20%. The authors concluded that allulose, but not fructose, led to modest reductions in the postprandial blood glucose response to oral glucose in individuals with type 2 diabetes.

Braunstein et al. (2018) assessed the effect of small single catalytic doses of fructose and allulose on postprandial blood glucose regulation in response to a 75 g OGTT in 27 healthy individuals in a randomised, crossover design with a minimum one-week washout between treatments. Two subjects dropped out after randomisation, so 25 subjects completed the trial. The treatments consisted of a standard 75 g-OGTT with the addition of fructose at 0 (control), 5, or 10 g; or allulose at 0 (control), 5, or 10 g. Small doses of fructose or allulose did not show a significant effect on plasma glucose incremental AUC or other markers of postprandial glycemic responses. The authors interpreted that the low power to detect a significant difference due to high intra-individual variations was responsible for no significant effects of D-allulose on postprandial glycemic responses. No adverse effects of D-allulose were observed.

In summary, acute single dose studies generally showed that D-allulose had favorable effects in suppressing postprandial glycemic responses. All long-term and intermediate-term studies consistently showed that D-allulose consumption did not result in any adverse effects on glucose metabolism indicators. No significant differences in blood glucose, insulin, and $HbA_{1c}$ were observed between the D-allulose and placebo groups when healthy subjects consumed D-allulose at daily doses up to 15 g for 5 to 48 weeks. However, in the sub-group with borderline diabetes, the D-allulose groups had lower HOMA-IR values with no statistical significance, and differential value of glucose ΔAUC on OGTT between baseline and week 48 was significantly decreased in the high D-allulose group than in the placebo. The overall data indicate favorable effects of D-allulose on glucose metabolism.

*C.1.2.6.3 Effects of D-allulose on renal function indicators in humans*

Long-term (48 weeks) consumption of D-allulose at daily doses up to 15 g did not result in significant changes related to renal function indicators (blood urea nitrogen, creatinine, uric acid, urine specific gravity, urinary microalbumin, and urine pH) (Tanaka et al., 2020).
A 12-week human study (Han et al., 2018b) also found no significant differences in renal function indicators between the D-allulose and control groups when D-allulose was administered at daily doses up to 12 to 14 g for 12 weeks in overweight subjects. Levels of plasma albumin and creatinine, the indirect markers of renal function, along with total bilirubin and γ-gamma-glutamyltransferase were not significantly different among the three groups (Han et al., 2018b).

Hayashi (2010) also reported no treatment-related abnormalities in urine parameters, such as urine concentrations of protein, glucose, and urobilinogen after the 12-week administration of D-allulose at daily dose of 15 g/person.

Overall, it is concluded that D-allulose consumption, at daily doses up to 15 g for 12 to 48 weeks, did not result in any adverse effects on renal function indicators.

*C.1.2.6.4 Effects of D-allulose on hepatic function indicators in humans*

Tanaka et al. (2020) reported that long-term (48 weeks) effects of D-allulose (up to 15 g/day) on hepatic function indicators, such as AST, alanine aminotransferase (ALT), ALP, and γ-glutamyl transpeptidase (γ-GTP). There were no statistically significant differences in absolute values of these indicators among the groups at the end of the 48-week administration and 52-week follow-up timepoints. However, differential values at week 48 from the baseline were significantly decreased for ALT, ALP, and γ-GTP (high-dose vs. low-dose vs. control: ΔALT, -4.4 vs. -3.7 vs. +1.9 U/L, $P<0.01$; ΔALP, -29.0 vs. -28.1 vs. +8.4 U/L, $P<0.01$; Δγ-GTP, -12.1 vs. -2.3 vs. +2.5 U/L, $P<0.01$). D-allulose consumption reduced the fatty liver scores as measured by ultrasonography in a dose-dependent manner (average score, higher is worse: control vs. low dose vs. high dose: 2.4 vs. 2.0 vs. 2.0; $P<0.05$).

Han et al. (2018b) and Hayashi (2010) also reported no treatment-related abnormalities in blood levels of AST, ALT, γ-GTP, and/or ALP between the D-allulose and control groups when D-allulose was administered at daily doses up to 15 g for 12 weeks.

Overall, it is concluded that consumption of D-allulose, at daily doses up to 15 g for 12 to 48 weeks, did not result in significant differences in hepatic function indicator enzymes. However, the fatty liver scores and differential values for liver function enzymes (ΔALT, ΔALP, and Δγ-GTP at weeks 12 to 48 from the baseline) were significantly decreased. The data indicated that a longer-term consumption of D-allulose had favorable effects on liver function indicator enzyme levels and may reduce the fatty liver scores.

*C.1.2.6.5 Effects of D-allulose on lipid metabolism in humans*

*C.1.2.6.5.1 A 48-week study by Tanaka et al. (2020)*

There were no significant differences in total cholesterol (TC) and LDL-C among the D-allulose and placebo groups at any test points. When comparing changes from baseline, a transient significant increase in LDL-C were observed in the high-dose D-allulose groups only at week 8 compared with week 0 (153.7 vs. 142.9 mg/dL, $P<0.05$). However, these values gradually

reduced after that timepoint, and all the values were within normal ranges of LDL-C. Thus, it was not considered as a toxicological concern.

On the other hand, TC levels were transiently significantly decreased at week 24 compared to the baseline in the high-dose D-allulose group (220.9 vs. 232.8 mg/dL, P<0.05). No statistically significant differences were noted in differential values among the 3 groups at weeks 16 through 48. However, at the 52-week follow up timepoint, both differential values of TC and LDL-C were significantly lower in the high-dose allulose group compared to the control group (changes from the baseline, control vs. high-dose allulose group: ΔTC, 6.0 vs. -8.0 mg/dL, P<0.01; ΔLDL-C, 5.4 vs. -7.1 mg/dL, P<0.01).

Decreases in HDL-C were observed in the D-allulose groups. However, the authors interpreted it as a mechanism for anti-atherosclerosis that enhances reverse cholesterol transport by increasing HDL-C intake into hepatocytes. In addition, all the HDL-C values were within normal ranges. Thus, small decreases were not considered as toxicological concerns.

There were no significant differences in small and very small LDL-C, which is an important risk factor of atherosclerotic cardiovascular disease beyond LDL-C itself, between the D-allulose groups and the placebo group. Carotid intima-media thickness, an indicator of the degree of arteriosclerosis, did not vary in the D-allulose groups compared with the placebo group before and after D-allulose intake for 48 weeks.

For other biomarkers for atherosclerotic cardiovascular disease, such as tumor necrosis factor-α (TNF- α), malonaldehyde, modified-LDL, interleukin (IL)-6, total plasminogen activator inhibitor-1 (t-PAI-I), remnant-like particles cholesterol, and high-sensitivity C-reactive protein, the differential values did not indicate significant increases between the test groups although some significant changes were observed when compared with week 0.

The authors concluded that D-allulose consumption for 48 weeks did not affect the absolute risk of atherosclerotic cardiovascular disease. No treatment-related adverse events were reported.

*C.1.2.6.5.2 12-week studies*

Studies by Han et al. (2018b) and Hayashi (2010) reported that D-allulose had no adverse effects on blood lipid profiles and/or inflammatory biomarkers when D-allulose was consumed at daily doses up to 15 g for 12 weeks by healthy normal or overweight subjects. There were no differences in plasma lipids, such as TG, TC, and LDL-C, among the test and control groups. In addition, differential values between the baseline and week 12 had no significant differences in the allulose and placebo groups.

In summary, D-allulose consumption at daily doses up to 15 g for 12 to 48 weeks did not adversely affect the blood lipid profile and the absolute risk of atherosclerotic cardiovascular disease.

*C.1.2.6.6 Effects of D-allulose on body fat in humans*

Han et al. (2018b) reported that D-allulose consumption, at daily doses up to 14 g for 12 weeks, significantly decreased differential values (Δ) of body fat percentage and body fat mass, such as total abdominal fat (high dose vs. low dose vs. control: -21.31 vs. -14.31 vs. +0.11 $cm^2$; P<0.05) and subcutaneous fat areas (high dose vs. low dose vs. control: -20.59 vs. -7.26 vs. -0.67 $cm^2$; P<0.01), at week 12 from the baseline in healthy overweight or obese subjects. These changes

were not accompanied with significant differences in nutrient intake, plasma lipid profiles, biomarkers of liver and kidney functions, and inflammation among groups.

Kimura et al. (2017) examined the effects of a single ingestion of D-allulose on postprandial energy metabolism in 13 healthy participants (mean age of 35.7 years and body mass index of 20.9 $kg/m^2$) in a randomised, single-blind crossover design with a 1-week washout period. At 30 minutes after taking 5 g of D-allulose or 10 mg of aspartame without any sugar as a control, overnight-fasted participants ingested a standardised meal, and energy metabolism was evaluated by a breath-by-breath method. In the D-allulose-treated group, the AUC of fat oxidation was significantly higher than in the control group (10.5 vs. 9.6 kJ/4 h/kg bw; $P < 0.05$), whereas that of carbohydrate oxidation was significantly lower (8.1 vs. 9.2 kJ/4 h/kg bw; $P < 0.05$). Furthermore, plasma $\Delta$glucose levels were lower at 90 minutes in the D-allulose group than in the control group, but at no other times. No other parameters, such as resting energy expenditure, insulin, TC, or TG, were modified. The authors concluded that D-allulose enhances postprandial fat oxidation in healthy humans, indicating that it could help maintain healthy body weight, probably through enhanced energy metabolism.

In summary, D-allulose consumption resulted in favorable effects on body fat composition although the absolute body weight measures were not statistically different among the treatment and control groups.

Table C.1.2.6-1. Summary of studies evaluating the effects of D-allulose on gastrointestinal tolerance and safety in humans

| Subjects | Daily dosage | Length | Measurements | Results | Reference |
|---|---|---|---|---|---|
| 10 Healthy males and females | Up to 0.9 g/kg bw/d | 1 day x 6 times | Gastrointestinal symptoms; physical conditions | No gastrointestinal symptoms up to 0.5 g/kg bw/d for males and 0.6 g/kg bw/d for females | Iida et al., 2007 |
| Healthy males and females | Gradually increased in steps of 0.1 g/kg bw | 11 weeks | Gastrointestinal symptoms | No gastrointestinal symptoms up to 0.4-0.9 g/kg bw | Han et al., 2018b |
| 17 normal subjects | 15 g/d (divided into 3 doses) | 12 weeks | Gastrointestinal symptoms, hematology, clinical chemistry, liver and renal function indicators, and urine parameters. | No adverse effects on gastrointestinal symptoms, hematology, clinical chemistry, liver and renal function indicators, and urine parameters. | Hayashi et al., 2010 |

d=day

Table C.1.2.6-2 Studies evaluating the effects of D-allulose on glucose, lipid and energy metabolism, body composition and renal and hepatic function indicators in humans

| Subjects | Daily dosage | Length | Measurements | Results | Reference |
|---|---|---|---|---|---|
| Repeated dose studies | | | | | |
| 90 adults mean and women, 20-65 y, including borderline diabetes | 0, 5, or 15 g/d | 48 week-interven-tion, follow-up at week 52 | Risk factors for atherosclerotic cardiovascular disease (CVD); glucose metabolism indicators, liver function indicator enzymes, fatty liver scores, renal function indicators | ↓ΔAUC between baseline and week 48 (high-dose vs. low-dose vs. control: -4.7 vs. 8.3 vs. 19.1 mg.hr/dL, P<0.01 for high-dose); No significant difference in risk factors for atherosclerotic CVD between groups; Significant improvement in hepatic enzyme activities and fatty liver scores. ↓ΔALT, ΔALP, and Δγ-GTP (high-dose vs. low-dose vs. control: ΔALT, -4.4 vs. -3.7 vs. +1.9 U/L, P<0.01; ΔALP, -29.0 vs. -28.1 vs. +8.4 U/L, P<0.01; Δγ-GTP, -12.1 vs. -2.3 vs. +2.5 U/L, P<0.01). No significant changes related to renal function indicators (blood urea nitrogen, creatinine, uric acid, urine specific gravity, urinary microalbumin, and urine pH); ↓Average fatty liver scores (higher is worse: control vs. low dose vs. high dose: 2.4 vs. 2.0 vs. 2.0; P<0.05) | Tanaka et al., 2020 |
| 121 healthy normo- or overweight, 20-40 y | 8 or 14 g in beverage (divided into 2 doses) | 12 weeks | Body composition, nutrient intake, plasma glucose and lipid metabolism indicators, clinical chemistry including liver and renal function indicators | ↓ changes in body fat percentage (high-dose vs. low-dose vs. control: -1.01 vs. -1.03 vs. -0.27%); ↓ ΔBMI (-0.48 vs. -0.32 vs. -0.13 kg/m$^2$); body fat mass (-1.11 vs. -1.14 vs. 0.34 kg); ↓ Δtotal abdominal (-21.3 vs. -14.3 vs. 0.11 cm$^2$); Δsubcutaneous fat areas (-20.6 vs. -7.3 vs. 0.67 cm$^2$). No significant changes in blood lipid and glucose metabolism indicators, and biomarkers of liver and renal functions and inflammation. | Han et al., 2018a |
| Single dose studies | | | | | |
| 26 adult men and women, including borderline diabetes patients, mean age, 55 y | 5 g in tea | Single dose; 5 g D-allulose in 200 mL tea with standard meal | Plasma glucose and insulin levels | ↓ blood glucose level after the meal (D-allulose vs. control: 161.6 vs. 174.0 at 30 min; p<0.01, 173.6 vs. 180.5 mg/dL at 60 min, p<0.05), ↓AUC glucose (5,739 vs. 6,482 mg.min/dL, p<0.01) in all subjects, ↓ blood insulin level (42.1 vs. 48.5 uU/mL, P<0.05) at 30 min. | Hayashi et al., 2010 |
| 20 healthy adults, mean ages 26-30 y | 0, 2.5, 5, or 7.5 g D- | Single dose | 75 g maltodextrin tolerance test - glycemic and | ↓ plasma glucose AUC with 5 to 7.5 g (control, 2,138; 2.5 g, 1,847; 5 g, 1533; 7.5 g, 1,468 uU.min/mL; P<0.01 for 5 to 7.5 g compared to the control). | Iida et al., 2008 |

| Subjects | Daily dosage | Length | Measurements | Results | Reference |
|---|---|---|---|---|---|
| | allulose in beverage | | insulinemic responses | | |
| 24 type 2 diabetes, 18-75 y | 0 (control), 5, or 10 g | Single dose | 75 g OGTT | ↓ plasma glucose incremental area under the curve (iAUC) in a dose response manner: 10 g compared with 0 g had an 8% reduction in iAUC. | Noronha et al. (2018) |
| 25 healthy, 18-75 y | 0 (control), 5, or 10 g | Single dose | 75 g OGTT | No significant effect on plasma glucose iAUC or other markers of postprandial glycemic responses. | Braunstein et al. (2018) |
| 13 healthy men and women, mean age, 35.7 y | 5 g | Single dose | Energy metabolism, blood biochemistry (lipid and glucose metabolism indicators) | ↑AUC of fat oxidation vs. control group (10.5 vs. 9.6 kJ/4 h/kg bw; $P < 0.05$); ↓Carbohydrate oxidation (8.1 vs. 9.2 kJ/4 h/kg bw; $P < 0.05$); ↑Plasma free fatty acids for the D-allulose group compared to control at 180 (0.12 vs. 0.10 mEq/L, $P<0.05$), 210 (0.17 vs. 0.11 mEq/L, $P<0.01$) and 240 minutes (0.21 vs. 0.15 mEq/L, $P<0.05$); Plasma ⊿glucose levels were transiently lower at 90 minutes in the D-allulose group than in the control group (25 vs. 35 mg/dl, $P<0.05$), but at no other times; No other parameters, such as resting energy expenditure, insulin, total cholesterol, or triacylglycerol were modified. | Kimura et al., 2017 |

*Excluded single dose studies; ALP= alkaline phosphatase; ALT= alanine aminotransferase; AST = aspartate aminotransferase; bw= body weight; BMI= body mass index; d=day; γ-GTP= γ-glutamyltransferase.

*C.1.2.7 Safety assessment reports prepared by international agencies or other national government agencies*

*(addressing section 3.5.2.C.4.3 of the FSANZ Application Handbook)*

There are no safety reports prepared by international agencies (such as WHO) or by national government agencies.

## C.2 Safety of the production organism

This section addresses various guidelines of the Application Handbook relating to the safety of the D-allulose-3-epimerace enzyme, which is expressed in the source microorganism, *M. foliorum*. Guideline 3.3.2.C sets out requirements relating to safety of an enzyme processing aid. Guideline 3.3.2.D sets out additional information related to the safety of an enzyme processing aid derived form a microorganism. Guideline 3.5.2.C.6.1 sets out requirements relating to the safety of novel foods derived from a new source (this is in addition to the safety requirements for novel foods that are dietary macro-components, which is covered in section C.1 above). The safety information presented below is presented in the context of the source microorganism, which harbours the D-psicose 3-epimerase enzyme responsible for converting fructose to D-allulose.

A battery of safety studies demonstrates that *M. foliorum* SYG27B-MF is safe for use in the manufacture of D-allulose:

- whole genome sequence information on *M. foliorum* SYG27B-MF has been used to confirm the absence of known toxigenic or pathogenic genes, virulence factors and antibiotic resistance genes (section C.2.1);

- single dose and sub-chronic (90-day) animal oral toxicity studies did not identify any adverse effects at high dose levels of *M. foliorum* SYG27B-MF (3 g/kg bw/day and 2 g/kg bw/day respectively) (section C.2.2); and

- In vivo and in vitro tests demonstrated that *M. foliorum* SYG27B-MF did not induce chromosomal aberrations, is not mutagenic, does not produce biogenic amines, is not resistant to a variety of antibiotics (defined by the European Food Safety Authority) and does not evidence an ability to hydrolyse gelatin (sections C.2.3 and C.2.4).

All traces of *M. foliorum* SYG27B-MF are removed after D-allulose is produced, meaning that *M. foliorum* SYG27B-MF, including the D-allulose-3-epimerase enzyme, used in the production of D-allulose will not be present in foods containing D-allulose. That is, consumers will not be exposed to the production organism, including the enzyme processing aid, when consuming foods containing D-allulose.

*C.2.1 Whole Genomic Sequence Analysis of Production Microorganism*

The whole genome sequencing results show *M. foliorum* SYG27B-MF to contain one circular chromosomal DNA and one plasmid (Table. C.2-1); it was taxonomically identified as *M. foliorum* SYG27B-MF according to closest related neighborhood match.

Table C.2.1-1. Whole genome sequence overview of *M. foliorum* SYG27B-MF

| | |
|---|---|
| **Genome** | *Microbacterium foliorum* |
| **Taxonomy ID** | 104336 (*Microbacterium foliorum*) |
| **Domain** | Bacteria |
| **Taxonomy** | Bacteria; Terrabacteria group; Actinobacteria; Actinobacteria; Micrococcales; *Microbacteriaceae; Microbacterium; Microbacterium foliorum* |
| **Closest neighbour** | marine *Actinobacterium* PHSC20C1 |
| **Size (bp)** | [redacted] |
| **GC Content in the DNA** | [redacted] |
| **Number of Contigs** | 1 circular chromosomal DNA |
| **Number of Coding Sequences** | 3608 |
| **Number of RNAs** | [redacted] |

### *C.2.1.1 Possible toxigenic gene detection*

The genome sequences of *M. foliorum* SYG27B-MF were compared with the genome sequences of four well-known pathogens (*E. coli*, *Enterococcus*, *Listeria*, and *Staphylococcus aureus*). The virulence factors included *E. coli* Shiga toxin gene and S. aureus exoenzyme genes, host immune alteration or evasion genes, and toxin genes. No virulence factors were found in the genomic sequences of *M. foliorum* SYG27B-MF. The results showed that toxic or pathogenic genes associated with *E. coli*, *Enterococcus*, *Listeria*, and *S. aureus* were not present in the genomic sequences of *M. foliorum* SYG27B-MF (Figure 1). In addition, when comparing the virulence genes between *M. foliorum* SYG27B-MF and closely related microorganisms using VFDB, there was no gene related to adherence factors, iron uptake, and toxin genes in *M. foliorum* SYG27B-MF.

### *C.2.1.2 Antibiotic resistant gene detection*

Whole genome sequencing of the *M. foliorum* SYG27B-MF strain was performed, and the result was matched based on the genomic database on the “Resfinder” web program. Antibiotic resistance genes were shown to be absent in *M. foliorum* SYG27B-MF.

### *C.2.1.3 Conclusion:*

The purpose of whole genome sequencing was to verify whether *M. foliorum* SYG27B-MF is safe based on genetic analysis. After identifying the whole genome sequence of the test organism, the sequence was applied to the virulence finder and VFDB, and also put into ResFinder to find the antibiotic resistant gene. According to the whole genome sequencing information, *M. foliorum* SYG27B-MF showed negative results for the major toxicity genes in the virulence finder. Details are presented in Annex F (CCI).

*C.2.2 Animal oral toxicity studies*

This non-GMO production organism utilised in the manufacture of D-allulose is not mutagenic, genotoxic, or toxic. An acute toxicity study showed that a single dose of 3 g/kg bw, the highest dose tested, did not cause any treatment-related abnormalities in Sprague-Dawley rats. The $LD_{50}$ was determined to be far above 3 g/kg bw. A 90-day sub-chronic toxicity study determined the NOAEL of *M. foliorum* SYG27B-MF as 2,000 mg/kg bw/day, the highest level tested. Details of these studies are summarised below.

*C.2.2.1 Single-Dose Oral Gavage Toxicity Study of M. foliorum SYG27B-MF in Rats*

Acute oral toxicities of *M. foliorum* SYG27B-MF were studied in 8-week old Sprague-Dawley (SD) rats (n=5/group) (Kim et al., 2018). Test substance was administered by oral gavage at a single dose of 0 or 3 g/kg bw. Animals were observed for fourteen days to monitor changes in body weight and clinical signs. At the end of the study, animals were sacrificed and major organs were macroscopically examined.

No animal died during the 14-day observation period and no abnormal clinical signs were observed at any dose level. No significant difference in mean body weight was found among the test and control groups. No treatment-related abnormalities were observed in macroscopic examinations. The author concluded that lethal dose ($LD_{50}$) of *M. foliorum* SYG27B-MF was well above 3 g/kg bw, the highest dose tested.

*C.2.2.2 Sub-chronic toxicity study of M. foliorum SYG27B-MF in rats*

Ninety-day, repeated oral dose studies were conducted to evaluate the oral toxicities of *M. foliorum* SYG27B-MF in male and female SD rats. The test substance was orally administered to 7-week-old Sprague-Dawley rats (10 male and 10 female rats) at a daily dose of 0, 500, 1,000, or 2,000 mg/kg bw for 90 days (Kim et al., 2018). Toxicity parameters included general symptoms, body weights, feed intakes, urinalysis, electrolyte, hematology, and blood biochemistry. In addition, eye test, organ weights, gross necropsy examination, and histopathological examination were performed.

None of the animals died during the period of administration, and no treatment-related abnormalities were noted in any parameters tested. Based on the results of the 90-day repeated toxicity test of SYG27B-MF, the NOAEL was determined to be 2,000 mg/kg/day, the highest level tested, in both male and female rats.

*C.2.3 In vivo and in vitro tests*

*C.2.3.1 In vivo micronucleus test of M. foliorum SYG27B-MF in mice*

*M. foliorum* SYG27B-MF was tested for its ability to induce micronuclei in polychromatic erythrocytes (PCE) of the bone marrow of treated Imprinting Control Region (ICR) mice according to the OECD Guidelines. The doses used in the study were 0 (solvent control), 500, 1,000, and 2,000 mg/kg bw (Kim et al., 2018). The 25 mice, aged 8 weeks (weighing 34.9 ~ 36.8 g), were treated by oral gavage with *M. foliorum* SYG27B-MF dissolved in saline over 2 consecutive days. Mitomycin C (2 mg/kg bw) was administered as a positive control. Animals were observed for clinical signs and mortality for 24 hours post-dosing. All doses were well tolerated and no clinical signs were observed. Bone marrow cells were collected at 24 hours after dosing and evaluated the frequency of micronuclei.

No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes (MNPCE) in PCE were observed in any test substance groups compared with the negative control group. A significant increase in the incidence of MNPCE in PCE was observed in the positive control group compared with the negative control group. Body weights of mice were comparable among the groups before and after the treatment with the test substance. The data suggest that *Microbacterium foliorum* SYG27B-MF did not induce chromosomal aberrations and is non-clastogenic in either the presence or absence of metabolic activation.

*C.2.3.2 Bacterial reverse mutation test of M. foliorum SYG27B-MF*

*In vitro* bacterial mutagenicity assays were performed to evaluate the mutagenic potential of *Microbacterium foliorum* SYG27B-MF in 4 strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and 1 strain of *E. coli* WP2*uvrA* (pKM101) in the absence and presence of metabolic activation (Kim et al., 2018). The test item was prepared by suspending in sterile distilled water. For the concentration determination test, 5 different concentrations (0, 61.7, 185, 556, 1,670, 5,000 μg/plate) were set. As a result of the concentration determination test, no increase in the number of reverse mutation colonies or overt cytotoxicity was observed for any of the strains regarding all concentrations.

In the main test, no reproducible increase in the number of colonies was noted in the presence and the absence of a metabolic activator in all five strains (*Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP 2uvrA) (up to 5,000 μg/plate). In addition, no dose-response relationship was observed. The frequency of colony formation by reverse mutation in the positive control group was 2-10 times ($P<0.05$) higher than the solvent control group, in the absence or the presence of a metabolic activation; thus, the conditions in this study were considered appropriate.

The data show that the test substance, *M. foliorum* SYG27B-MF, was not mutagenic under the conditions used in this study.

*C.2.3.3 In vitro mammalian chromosome aberration test of M. foliorum SYG27B-MF*

The cytotoxicity of *M. foliorum* SYG27B-MF and its potential to induce chromosomal aberrations were assessed in Chinese hamster ovarian fibroblasts (CHO-K1 cell) in the presence or absence of metabolic activation (Kim et al., 2018). The test substance was prepared by suspending it in sterile distilled water.

In a dose range test, seven different concentrations (80, 160, 320, 630, 1,250, 2,500, and 5,000 μg/mL) of the test substance were used to measure the inhibition of cell proliferation (as measured by cell number per concentration, and the rate of inhibition of proliferation). The inhibition of cell proliferation by more than 50% was not found in any of the concentrations in the presence and the absence of S9 (rat liver homogenate) at 6 and 24 h. Therefore, in the main experiment, three test concentrations of 1,250, 2,500, and 5,000 μg/mL were chosen. All three test groups did not show significant differences in the numerical or structural chromosomal anomalies compared to the solvent control. Positive controls (cyclophosphamide monohydrate and Mitomycin C) showed significant differences in numerical and structural chromosomal anomalies; thus, the conditions in this study were considered appropriate.

The author concluded that the test substance, *M. foliorum* SYG27B-MF did not cause numerical or structural chromosomal anomalies in these experimental conditions.

Biogenic Amine Analysis

The presence of microbial bioamine was investigated in the allulose products (syrup with allulose >8% and >62% and powder with allulose >98%) by high-performance liquid chromatography (HPLC). The analytes included tryptamine, 2-phenylethylamine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, histamine dihydrochloride, 1,7-diaminoheptane, tyramine hydrochloride, spermidine trihydrochloride, and spermine tetrahydrochloride. No bioamine was detected in all products. More detail is provided in Annex K (CCI).

### *C.2.4 Tests for antibiotic resistance and gelatin hydrolysis*

#### *C.2.4.1 Antimicrobial Susceptibility Test against M. foliorum SYG27B-MF*

Annex F (CCI) presents the results of an *in vitro* antimicrobial susceptibility test on *M. foliorum* SYG27B-MF. The experiment was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, and the antibiotic resistance break-points applied according to the European Food Safety Authority (EFSA 2019). *M. foliorum* SYG27B-MF was considered under the criteria for *Corynebacterium* spp. in M45 of CLSI guideline. A pure culture of *M. foliorum* SYG27B-MF was cultivated at 30°C for 24 h in allulose broth, and the quality control (QC) strain (*S. pneumoniae* ATCC49619) was cultivated at 37°C for 24 h in CAMHB-LHB (5%) media. The broth dilution method was used to assess the minimal inhibitory concentration (MIC) of the strain against antibiotics.

In the broth microdilution, a pure culture of each test organism was cultivated in broth, and then washed with sterilised phosphate-buffered saline (PBS). The washed bacterial solution was adjusted to 0.01 – 0.02 of 600 nm optical density (OD) units in PBS. Ten microlitres (1-2 x $10^5$ CFU/mL) of the strain was inoculated in 96 well plates containing 200 µL of CAMHB-LHB broth medium with antibiotics. Experimental results are visually confirmed, and the antibiotic concentration at the point where bacteria do not grow is used as the MIC value.

The broth dilution method was used to evaluate the minimal inhibitory concentration (MIC) for each antibiotic. *M. foliorum* SYG27B-MF was found to be sensitive to all the tested antibiotics according to the MIC breakpoints suggested by EFSA for *Corynebacterium* and other Gram-positive bacteria. *S. pneumoniae* ATCC49619, which was used as a control QC strain, showed that the results were within the QC range based on the CLSI, although with slightly higher values for gentamicin, streptomycin, and kanamycin. The determined MIC values were clearly below or equal to the recommended EFSA breakpoint values (Table C.2.4.1-1).

Table C.2.4.1-1. Results of broth dilution MIC test for *M. foliorum* SYG27B-MF

| **Antibiotic resistance test** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Strain** | **Minimum inhibitory concentration (mg/L) of antibiotics** | | | | | | | | |
| | **Amp** | **Ery** | **Gen** | **Tet** | **Str** | **Chl** | **Cli** | **Kan** | **Van** |
| *S. pneumoniae* ATCC49619 | 0.125 | 0.5 | 16 | 0.125 | 32 | 2 | 0.125 | 32 | 0.25 |
| *M. foliorum* SYG27B-MF | 0.5 | 0.5 | 4 | 2 | 1 | 2 | 0.125 | 4 | 0.5 |
| **EFSA breakpoint** | **1** | **1** | **4** | **2** | **8** | **4** | **4** | **16** | **4** |

*Amp = Ampicillin, Ery = Erythromycin, Gen = Gentamicin, Tet = Tetracycline, Str = Streptomycin, Chl = Chloramphenicol, Cli = Clindamycin, Kan = Kanamycin, Van = Vancomycin

*C.2.4.1.1 Conclusion*

This experiment was conducted to determine whether the test bacterium, *M. foliorum* SYG27B-MF, is resistant to nine antibiotics as defined by EFSA. When the genome of the test organism was searched through whole genome sequencing, no antibiotic resistance gene was detected in the test organism. In addition, the physiological susceptibility level of the test organism was measured when exposed to antibiotics. The test method followed the CLSI guideline including the QC strain used as designated by CLSI. In addition to information on the antibiotic resistance genes, results of the *in vitro* susceptibility test on the nine antibiotics showed that the resistance of the test strain did not exceed the EFSA standard. Thus, the test organism, *M. foliorum* SYG27B-MF, can be considered as safe for use with regard to antibiotic resistance.

*C.2.4.2 Gelatin Hydrolysis Assay of M. foliorum SYG27B-MF*

The gelatin hydrolysis test is used to detect the ability of an organism to produce gelatinase (proteolytic enzyme) that liquefy gelatin. Gelatin is a protein derived from collagen, the connective tissues of vertebrates. The enzyme gelatinase can hydrolyse gelatin and an extracellular zinc metalloprotease that has been shown to potentially contribute to the virulence of *Enterococcus faecalis* in some animal models (Annex F - CCI). This, the production microorganism's ability to hydrolyse gelatin was determined.

*M. foliorum* SYG27B-MF, grown at 30°C for 24 h in allulose broth, was inoculated with a needle in a gelatin medium and incubated at 30°C for up to 5 days, and checked daily for gelatin liquefaction and bacterial growth. Gelatin normally liquefies at 28°C and above. To confirm that liquefaction was due to gelatinase activity, the tubes are immersed in a refrigerator for 30 minutes. Afterwards, tubes are tilted to observe if gelatin has been hydrolysed. Hydrolysis of gelatin will result in a liquified medium even after exposure to cold temperature. *Bacillus cereus* ATCC 11778 was used as a positive control. *M. foliorum* SYG27B-MF showed a negative reaction for gelatin hydrolysis test. On the other hand, *B. cereus* ATCC 11778, used as a positive control, degraded gelatin, rendering the medium liquid like a broth medium. These experiments confirm that *M. foliorum* SYG27B-MF does not show the ability to hydrolyse gelatin, indicating that the production microorganism is not likely to possess mucolytic activity. Details are provided in Annex F (CCI).

### *C.2.4.3 Test bacteria growth condition*

*M. foliorum* SYG27B-MF was incubated at 30°C under aerobic conditions in the allulose medium provided by Samyang.

The reference strains, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Enterococcus faecalis* ATCC29212, and *Bacillus subtilis* ATCC6633, were cultured on Mueller Hinton Agar (MHA) agar and in Cation-Adjusted Mueller Hinton Broth (CAMHB) medium. They were incubated at 30°C for 24 h under aerobic conditions. Media composition is presented in Table C.2.4.3-1.

Table C.2.4.3-1 Composition of allulose medium for *M. foliorum* SYG27B-MF growth

| | |
|---|---|
| Allulose | 5g/L |
| Yeast extract | 2g/L |
| $(NH4)_2SO_4$ | 7g/L |
| $KH_2PO_4$ | 0.5g/L |
| $K_2HPO_4$ | 0.5g/L |
| $MgSO_4.7H_2O$ | 0.5g/L |
| $MnSO_4.4H_2O$ | 4.2mg/L |
| $FeSO_4.7H_2O$ | 6mg/L |
| Biotin | 0.2mg/L |
| Thiamine chloride | 0.2mg/L |
| pH | 6.8 |

#### *C.2.4.3.1 Double layer antagonistic activity test*

Since the growth conditions for *M. foliorum* SYG27B-MF were not favourable for co-cultivation with the 5 strains of the reference group, a double layer agar plate was used to perform the antagonistic activity test. Only a few colonies of the reference strains were observed in the allulose media for growing *M. foliorum* SYG27B-MF. The MHA medium was poured 3mm thick into a 60mm culture dish. The day before the experiment the reference strains incubated in CAMHB were collected, washed with PBS, and further diluted with PBS to adjust the O.D value to 0.01. 60 μL of the diluted bacterial solution was spread evenly on the surface of the agar medium. After 2 h, 1.5% sterilised pure agar was poured 1mm thick on the inoculated bacteria. After one day incubation, growth of the reference strains was investigated. The allulose medium was poured evenly on the 1.5% pure agar to 3mm thickness. After this, the allulose medium was inoculated with *M. foliorum* SYG27B-MF and incubated for 3 days. The clear zone formation and the growth of the reference strains and test bacterium (*M. foliorum* SYG27B-MF) was investigated.

#### *C.2.4.3.2 Culture supernatants antagonistic activity test*

One day before the experiment, *M. foliorum* SYG27B-MF was incubated in allulose broth, and 5 reference strains were cultured in CAMHB. On the day of the test, the culture solution was centrifuged for 1 minute at 15,000 rpm. The five reference strains were diluted to 1 X $10^9$ CFU/mL using sterile PBS after centrifugation. 50μL of the diluted bacterial solution of 5 reference strains was added to 5mL of CAMHB. Immediately after the addition, 500μL of PBS was added to one group, 500 μL of allulose broth was added to the other group, and 500 μL of *M. foliorum* SYG27B-MF cultured supernatant was added to the last group. All groups were checked for living bacteria after 24 hours of incubation at 37°C.

*C.2.4.3.3 Results*

The five reference strains and *M. foliorum* SYG27B-MF grew well individually without affecting each other's growth. In the picture, the growth of *E. faecium* and *B. subtilis* appeared not to be observed. However, in the cultured plate, the growth of the bacteria was detected.

The results of the effect of the produced *M. foliorum* SYG27B-MF substances on the growth of other reference strains are presented in Annex F (CCI). The growth of the reference strain did not differ from the control even in the group containing 500 µL of the *M. foliorum* SYG27B-MF cultured supernatant.

*C.2.4.3.4 Conclusion*

In this experiment, we confirmed that *M. foliorum* SYG27B-MF produces a substance that can inhibit the growth of other bacteria. When the five reference strains used in the test were cultured in allulose medium supporting the growth of *M. foliorum* SYG27B-MF, the slow growth of the bacteria was observed. This could be due to the large amount of allulose contained in the medium. Since *M. foliorum* SYG27B-MF and the reference strains could not grow under the same medium conditions, a double layer antagonistic activity test was carried out using agar media where each organism could grow. As a result, it was observed that all the bacteria grew well in the medium without interfering with each other's growth. When antibiotics were dropped on the *M. foliorum* SYG27B-MF-inoculated layer as the positive control, the growth of the reference strains was inhibited, and clear zones were formed (data not shown). In addition, it was confirmed that no substance inhibiting the growth of reference strain was present in the culture medium of M. foliorum SYG27B-MF. In summary, it could be confirmed that the test bacterium, *M. foliorum* SYG27B-MF, did not release substances that inhibit the growth of other bacteria.

*C.2.5 Allergenicity considerations*

There is no residual production microorganism in the D-allulose preparation. Total protein content is less than 0.2%. It is not likely that D-allulose causes allergenic reactions.

The amino acid sequence of D-psicose 3-epimerase is shown below:

[redacted]

A search for similarity of amino acid sequence of the enzyme to known allergens was made, and no match was found.

In addition, the production microorganism did not elicit any allergenic reactions in rats. *M. foliorum* SYG27B-MF, the test substance, was repeatedly administered to 40 Sprague-Dawley rats at a dose of 0, 500, 1,000, and 2,000 mg/kg bw for 90 days. Serum immunoglobulin (Ig) E level was measured by enzyme-linked immunosorbent assay (ELISA). The serum IgE levels in the male rats were not significantly different among the test and control groups. In the high-dose group (2,000 mg/kg), the serum IgE levels tended to decrease compared to the control group (0 mg/kg) at each measurement, but it was not significant since it was within the deviation range.

The serum IgE levels in the female rats of the low-dose group (500 mg/kg bw) tended to increase to 26.2 ± 5.46 ng/mL compared to the control group (0 mg/kg; 16.5 ± 2.89 ng/mL). However, the mid-dose, high-dose, and control groups had comparable IgE levels. Thus, it is concluded that *M. foliorum* SYG27B-MF did not elicit allergenic reactions. Details are shown in Annex K.

### *C.2.6 Information on the genetic stability of the source organism*

*(addressing section 3.3.2.D.3 of the FSANZ Application Handbook)*

Information on the cultivation of *M. foliorum* SYG27B-MF is provided in Annex J (CCI). The cultivation process applies the same conditions consistently between batches. Samyang observes morphology, growth and production characteristics of M. foliorum SYG27B-MF across batches. An example of observations across three batches of cultivated production strain is provided below in Table C.2.6-1 and demonstrates consistent morphology, growth and activity (in terms of converting fructose to D-allulose).

Table C.2.6-1 Cell mass, activity, and morphology across 3 batches of *M. foliorum* SYG27B-MF

| Batch # | Culture time (hr) | Cell mass (g*/L) | Cell activity (U**/g) cell | Morphology |
|---|---|---|---|---|
| 1 | 47 | 13.6 | [redacted] | Light yellow colour, rod-shaped bacterium |
| 2 | 47 | 12.6 | [redacted] | Light yellow colour, rod-shaped bacterium |
| 3 | 47 | 13.3 | [redacted] | Light yellow colour, rod-shaped bacterium |

* 'g' is the value of the dry cell mass measured by substituting the dry cell mass conversion factor of 0.35 for the absorbance (600 nm) value measured from the culture broth

** Cell activity (U/g_cell): One unit means the production of allulose (mM) converted from fructose per one gram of cells for one minute

## D Information on the dietary exposure to the novel food

*(addressing section 3.5.2.D of the FSANZ Application Handbook)*

The intended use of D-allulose in foods is described in section D.1. The natural presence of D-allulose in foods is described in section D.2. A dietary intake assessment based on US consumption data is described in Section D.5. The application does not include responses to the Application Handbook guidelines 3.5.2.D.3 and D.5 because the intended uses of D-allulose are in commonly consumed foods that are represented in the most recent national nutrition surveys of Australia and New Zealand and the consumption of these foods is unlikely to have changed significantly in recent years.

### D.1 Intended use of D-allulose in foods

*(addressing sections 3.5.2.D.1 and D.2 of the FSANZ Application Handbook)*

The intended uses of D-allulose as a novel food in Australia and New Zealand are listed in Table D.1-1. The use levels in column two are maximum use levels.

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

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

| Food category | Maximum use levels % (w/w) |
|---|---|
| Bakery products (bread rolls, cakes, cake-type rolls, pastries, doughnuts, biscuits (including cookies, shortbread, butter milk and whole wheat biscuits, crackers)); reduced energy | 10 |
| Beverages (water based, non-alcoholic); low- and reduced energy, low- and reduced sugar (including sweetened teas, instant coffees but not including cereal/nut/legume-based milk analogues) | 3.5 |
| Breakfast cereals and cereal based bars; regular | 2 |
| Breakfast cereals and cereal bars; reduced energy; reduced sugar | 5 |
| Chewing gum | 50 |
| Icings and frostings | 5 |
| Frozen dairy desserts (ice cream, soft serve, sorbet); low- and reduced-energy and low- and reduced sugar | 5 |
| Yogurt and frozen yogurt; low- and reduced energy; low- and reduced sugar | 5 |
| Dressings for salads | 5 |
| Gelatins, pudding and fillings; low- and reduced energy, low- and reduced sugar | 10 |
| Hard candies/confectionery; low- and reduced energy | 50 |
| Soft candies/confectionery; low- and reduced energy (not including chocolate) | 25 |
| Jams and jellies | 10 |
| Sugar products | 10 |
| Sugar substitutes | 100 |
| Sweet sauces and syrups; low- and reduced- energy, low- and reduced sugar | 10 |
| Fat-based cream (used in modified fat/energy cookies, cakes, pastries, and pie) | 5 |

Samyang does not intend to use D-allulose as an ingredient in infant formula products, formulated supplementary foods for young children or in raw commodity products such as fresh meat, fruit and vegetables.

### D.2. Natural presence of D-allulose in foods

*(addressing sections 3.5.2.D.1 and D.2 of the FSANZ Application Handbook)*

Table D.2-1. Natural presence of D-allulose in foods (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 |

### D.3 The percentage of the food group in which the novel food ingredient is proposed to be used or the percentage of the market likely to use the novel food ingredient

*(addressing section 3.5.2.D.4 of the FSANZ Application Handbook)*

D-allulose is intended to be added to reduced energy and reduced sugar foods within a number of the categories identified above in Table D.1-1. It is unlikely that all foods within the categories identified in Table D.1-1 will contain added D-allulose if the ingredient is approved. Samyang is an ingredient manufacturer and supplier and it is difficult to estimate potential market share or

uptake of D-allulose in the Australian and New Zealand manufactured food sector. Samyang considers that 10% market share as an alternative to other sugar ingredients like sucrose will be a strong achievement, particularly in the short to medium term.

## D.4 Data to show whether the food, or the food in which the novel food ingredient is used, is likely to replace another food from the diet

*(addressing section 3.5.2.D.4 of the FSANZ Application Handbook)*

D-allulose is intended to be used as a substitute for sugar and other carbohydrate ingredients in foods. Rather than replacing other foods in the diet, D-allulose containing foods will be alternative options to existing foods that contain sugars, such as sucrose, as ingredients. Therefore, Samyang considers it unlikely that the use of D-allulose as an ingredient in foods will replace foods in the diet. Foods containing added D-allulose will be alternatives to the same foods containing conventional sugars like sucrose.

## D.5 Dietary exposure estimates for US population

Based on the intended uses in Table D.1-1, estimates of dietary intake (EDI) to D-allulose have been made using US national nutrition survey food consumption data. Although, FSANZ will conduct a dietary exposure assessment based on food consumption data from the latest national nutrition surveys in Australia and New Zealand, the estimated dietary intakes (EDIs) based on US data provide a valuable comparison and a level of assurance that the intended uses of D-allulose are safe.

### *D.5.1 EDI of D-allulose under the intended use*

Based on the food consumption data reported in a recent NHANES (2011-2014) dataset compiled by the US Department of Health and Human Services, National Center for Health Statistics, and the Nutrition Coordinating Center, the EDIs of D-allulose under the intended use of Samyang's D-allulose were calculated from the food code list and the survey database of diet recalls.

The results of the EDI assessment are summarized in Tables D.5.1-1 and D.5.1-2. Table D.5.1-1 presents the results of the mean of the population as well as the 90$^{th}$ percentile in g/day, and Table D.5.1-2 in g/kg bw/day. The mean and 90$^{th}$ percentile EDIs of all users aged 2 years and older were 11.0 and 30.0 g/person/day, respectively. All users had EDIs equal to or below 0.5 g/kg bw/day. These results reveal an average maximum exposure would occur in males older than 19 years of age, with a 90$^{th}$ 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.

These estimates are based on the conservative assumption that D-allulose will be added at the maximum use level in all food categories listed in Table D-1. The estimates are therefore over-estimated since it is not likely that D-allulose will be used at the maximum levels for all food categories under the intended uses. Overall, the intended use will result in EDIs at levels significantly below those associated with any potential side effects.

Table D.5.1-1. Maximum EDIs of D-Allulose, g/day*

| Population | N-user* | Per User (g/day) | | Per Capita (g/day) | |
|---|---|---|---|---|---|
| | | Mean | 90th Percentile | Mean | 90th 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 2011-2014. U.S.= United States

Table D.5.1-2. Maximum EDIs of D-Allulose, g/kg bw/day*

| 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 2011-2014. BW=body weight.

*D.5.2 EDI 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), 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 the same as those described in GRN 693. These values are comparable to the EDI value of 206 mg/person/day, which was reported by Oshima et al. (2006) by assuming a daily diet consisting of fruit, cereal, fruit juice, Bolognese spaghetti, crème caramel, coke, hamburger, and fruit cocktail. These estimates of dietary intake of naturally occurring D-allulose from the diet are significantly less than the potential intake from the intended uses of D-allulose described above. Data from Samyang's estimated dietary exposure to naturally occurring D-allulose from the diet are presented in Table D.5.2-1 (all users or consumers of D-allulose containing foods) and Table D.5.2-2 (total population).

Table D.5.2-1. 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=90$^{th}$ percentile; Based on NHANES 2011-2014.

Table D.5.2-2. 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=90$^{th}$ percentile; Based on NHANES 2011-2014.

*D.5.3 Summary of exposure estimates*

Among consumers in the total population aged 2 years and older, the mean and 90$^{th}$ percentile of all-user intakes of D-allulose were determined to be 11.0 and 30.0 g/person/day, respectively, under the intended use when the 2011-2014 NHANES dataset was used to calculate the EDIs. Males older than 19 years of age would have the highest 90$^{th}$ percentile intake among the various age/gender groups, with the 90$^{th}$ percentile value of 36.3 g/person/day in all-users. On a body weight basis, children aged 2-12 years had the highest 90$^{th}$ percentile EDI at 0.5 g/kg bw/day in all-users. Compared to EDIs under the intended use, exposure to D-allulose from the diet is negligible; the mean and 90$^{th}$ percentile EDIs from the diet were estimated to be 94.8 and 260.7 mg D-allulose/person/day in all users.

The EDIs presented above are over-estimates since it is not likely that D-allulose will be used at the maximum levels for all food categories under the intended uses. In addition, short-term surveys, such as the typical 2-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently.

## D.6 Dietary exposure of the processing aid

The Application Handbook requires information on dietary exposure to processing aids (section 3.3.2.F), including residues of processing aids or metabolites in foods containing the ingredient that was prepared by the use of the processing aid. However, Samyang notes that the production organism (M. foliorum SYG27B-MF) is not present in Samyang's D-allulose ingredient that is intended to be consumed and added to foods as an ingredient. By virtue of the absence of the organism, the enzyme, which is housed within the organism, is also not present in Samyang's D-allulose ingredient. Given the absence of the organism and therefore the enzyme in the D-allulose ingredient, there is no dietary exposure to the processing aid expected via the consumption of D-allulose. Therefore, Samyang does not consider the requirements of section 3.3.2.F of the Application Handbook are applicable for this application.

Samyang commissioned a study to determine if residues of the *M. foliorum* SYG27B-MF harbouring the enzyme D-allulose-3-epimerase were present in Samyang's final D-allulose ingredient intended for use in foods. The full report for this study is provided at Annex E (CCI). A brief summary is provided below.

Three products samples (one of each of Samyang's two liquid D-allulose products and one of the powdered D-allulose product) from five different batches were tested to determine whether there were any detectable levels of live bacteria present and whether any DNA fragments of *M. foliorum* SYG27B-MF were present. Viable cell counts were conducted after incubating samples of all batches for six days, with no detections of the organism (measured value of 10 CFU/mL). Polymerase chain reaction analysis of all batch samples using specific primers for M. foliorum SYG27B-MF was conducted, with no detections of DNA from the organism observed (at the detection limit of 10 ng of DNA per g or mL of sample).

This study provides clear evidence that no residues of the M. foliorum SYG27B-MF organism (and therefore the D-allulose-3-epimerase enzyme that is present in the organism) are present in the final D-allulose ingredient.

# E Information on the nutritional and health impact of the novel food

*(addressing section 3.5.2.E of the FSANZ Application Handbook)*

## E.1 Information to demonstrate that the use of the novel food or novel food ingredient will not cause a nutritional imbalance in the diet

*(addressing section 3.5.2.E.1 of the FSANZ Application Handbook)*

D-allulose is intended to be used as a substitute for sugar in food products listed in Table D.1-1. The consumption of added sugars is a public health concern, particularly in relation to excess energy consumption. The replacement of conventional sugar ingredients (such as sucrose) with a low-energy alternative such as D-allulose may play a role in reducing the intake of conventional added sugars. Samyang considers that D-allulose containing foods are likely only to replace conventionally sweetened counterpart foods rather than substituting for other foods or food groups. This may result in a reduction in the consumption of conventional sugars among consumers who consume D-allulose containing foods.

D-allulose is not intended to replace any other type of ingredient, such as fats or dietary fibres, in food products – other than conventional sugars. D-allulose is intended to be used as an alternative ingredient to conventional sugars, offering reduced energy content because of the removal of conventional sugars and substitution by D-allulose. Samyang is not aware of data to demonstrate that consumers may substitute D-allulose containing foods for other types of foods that may result in a nutritional imbalance in the diet (based on the literature search approach outlined in section 4 of the application).

Section C of the application highlights the rapid absorption and excretion of the majority of D-allulose in urine after oral administration. Samyang has not identified studies other than those cited in this application that have investigated potential impacts of D-allulose on the absorption of other nutrients in the diet. D-allulose is therefore not expected to affect the bioavailability of other ingredients in foods, similar to sucrose and other simple sugar-type ingredients, particularly monosaccharides such as glucose and fructose.

## E.2 Information to demonstrate that the addition of the novel food ingredient will not create a significant negative public health impact

*(addressing section 3.5.2.E.2 of the FSANZ Application Handbook)*

The Application Handbook states that if the purpose of addition of a novel food ingredient relates to a potential beneficial physiological or health-related outcome, information from scientific studies on any potential adverse effect(s) on the physiological status of the target or non-target population should be provided. Samyang notes the purpose of addition of D-allulose to foods is as a low-energy sugar replacement ingredient, which will provide foods with lower energy content compared to foods containing conventional sugar sweeteners, such as sucrose. Samyang considers that reducing the energy and conventional sugar content of foods by using D-allulose in place of conventional sugars is not related to a specific potential beneficial physiological or health related outcome.

However, energy consumption, particularly from added sugars, is an important public health issue in Australia and New Zealand. The availability of foods with safe, lower energy content ingredients in place of sucrose and similar simple carbohydrates is likely to provide a positive public health impact in the context of the consumption of added sugars and energy intake.

Again, the safety information provided in section C of this application demonstrates that D-allulose is well tolerated and safe for consumption at the intended use levels. Human studies have not identified adverse effects associated with D-allulose intake at the intended levels of use listed in section D of this application.

## F Information related to potential impact on consumer understanding and behaviour

*(addressing section 3.5.2.F of the FSANZ Application Handbook)*

### F.1 Information to demonstrate the level of consumer awareness and understanding of the novel food or novel food ingredient

*(addressing section 3.5.2.F.1 of the FSANZ Application Handbook)*

If approved, D-allulose will be marketed as a low energy sugar substitute ingredient in reduced or lower energy food products. Claims about lower energy content are regulated by the Code; foods containing D-allulose will be required to comply with these requirements, including nutrition information requirements (discussed in section G). As is the case with unapproved novel foods generally, consumer awareness of D-allulose at present in Australia and New Zealand is limited because the ingredient is not permitted. Once D-allulose is approved it may take some time for consumers to become aware of D-allulose as a potential lower energy sugar substitute ingredient. Consumers who are searching for lower energy food products may choose alternatives that contain D-allulose, based primarily on the labelling requirements for lower energy foods and nutrition content claims for sugar (see section G.3 for more detail on nutrition content claims for sugar). Associated marketing of D-allulose by food manufacturers or retailers (for example, on industry websites) may also increase consumer awareness and understanding of the low energy properties of D-allulose.

### F.2 Information on the actual or potential behaviour of consumers in response to the novel food or novel food ingredient

*(addressing section 3.5.2.F.2 of the FSANZ Application Handbook)*

The intended purpose of adding D-allulose to foods is as a low energy sugar substitute ingredient. Samyang expects that manufacturers of foods containing added D-allulose as a substitute for conventional sugars will market the lower energy content of the foods and may also focus on the decreased conventional sugar content (sugar nutrition content claims are discussed in section G.3). As noted above, Samyang is not aware of information on consumer behaviour in relation to D-allulose. However, labelling of foods containing D-allulose may appeal to consumers that are targeting lower energy foods. Label claims, such as nutrition content claims about energy and/or sugar are controlled by the Code. Foods containing added D-allulose will cost more than conventionally sweetened alternatives because D-allulose costs more to produce than sugars such as sucrose. Some consumers may not be willing to purchase foods containing added D-allulose on the basis of increased cost.

### F.3 Information to demonstrate that the foods containing the novel food ingredient will not adversely affect any population groups

D-allulose is a low-energy sugar replacement ingredient that is intended to be added to a variety of foods available to the general population. The replacement of conventional sugar, such as sucrose, with D-allulose will not adversely affect any population groups. This application has demonstrated that D-allulose is safe for consumption at the intended use levels described above in section D.

## G Nutrition labelling information

*(addressing section 3.2.5.A and B and of the FSANZ Application Handbook)*

D-allulose is intended to replace sugar as an ingredient in foods. D-allulose is not metabolised like other simple sugars in animals and humans, meaning that significantly less energy is available from the consumption of D-allulose compared to commonly used sugars, such as sucrose. The lower energy content of D-allulose compared to other sugars needs to be reflected in nutrition labelling for foods that will contain D-allulose as an ingredient (see Table D.1-1 for a list of foods intended to contain added D-allulose).

Schedule 11 of the Code includes requirements for calculating the average energy content of foods for nutrition labelling purposes. Generic energy factors apply for available carbohydrate (17 kJ/g) and unavailable carbohydrate (such as dietary fibre) (8 kJ/g) and for protein (17 kJ/g), fat (37 kJ/g), and alcohol (29 kJ/g). However, these generic energy factors are not appropriate for ingredients that provide different levels of energy. Subsection S11—2(3) of the Code lists ingredients that have different energy factors. Samyang is requesting D-allulose be added to this list of ingredients, with an energy factor of 1.0 kJ/g, to ensure that accurate values for the energy content of foods containing D-allulose can be included on labels. The calculation of the energy factor for D-allulose is addressed in section G.1.

The inclusion of an energy factor for D-allulose in S11—2(3) of the Code will also impact on other aspects of nutrition information labelling, including the determination and labelling of carbohydrate content and claims relating to sugar content. Section G.2 addresses the general nutrition labelling requirements of the Application Handbook in the context of how a new energy factor may impact on carbohydrate and sugar labelling of foods containing D-allulose.

Section G.3 of the application addresses Samyang's request for D-allulose to be treated differently to other sugars in the context of making nutrition content claims about sugar(s). Section G.3.1 sets out Samyang's request and justification for D-allulose to be treated differently to conventional sugars. Section G.3.2 addresses the Application Handbook's section 3.2.6.A.1 requirements for nutrition content claims amendments.

### G.1 Details on the calculation of the energy factor

*(addressing section 3.2.5.B of the FSANZ Application Handbook)*

Guideline 3.2.5.B of the FSANZ Application Handbook sets out requirements for calculating a new energy factor. Guideline 3.2.5.B.1 requires information on the nature and composition of the food ingredient, which is provided in the context of structure/properties (section B.3) and impurities and specifications (sections B.4 and B.6 respectively). The safety of D-allulose and its

production organism is discussed in sections C.1 and C.2, respectively. The remainder of the Guideline 3.2.5.B requirements are addressed below.

The Application Handbook specifies the energy factor for a food ingredient to be calculated according to the following equation, noting that other calculation methods will not be considered:

**ME = GE - FE - UE - GaE - SE**

where
**ME** means **metabolisable energy**
**GE** means **gross energy** (as measured by bomb calorimetry).
**FE** means energy lost in **faeces**.
**UE** means energy lost in **urine**.
**GaE** means the energy lost in **gases** produced by fermentation in the large intestine.
**SE** means the energy content of waste products lost from **surface areas**.

The following values have been assigned to the elements of the equation for D-allulose:

GE = 15.2 kJ/g (based on the value for fructose previously identified by FSANZ (D-allulose is an epimer of fructose))
FE = 0 - 0.68 kJ/g (based on an assumption of zero up to a value based on average of range (2-6% = average of 4%) reported in Williamson et al. (2014))
UE = 12.67 kJ/g (based on average of range in urine at different dose levels of D-allulose (reported by Iida et al. (2010))
GaE = 0.85 (based on the default value in the Application Handbook for ingredients fermented or partly fermented in the large intestine)
SE = 0 (based on the default value in the Application Handbook)

Samyang has used two values in estimating FE in the above equation. The reasoning is explained below and in more detail in section G.1.1. This results in two values for ME, depending on which value of FE is used, 1.0 kJ/g or 1.68 kJ/g.

| **ME value** | Equation (ME = GE – FE – UE – GaE – SE) |
|---|---|
| **1.0** | 15.2 – 0.68 – 12.67 – 0.85 – 0 |
| **1.68** | 15.2 – 0 – 12.67 – 0.85 – 0 |

Samyang considers the lower value is appropriate and is more likely to be representative of human faecal excretion rates of D-allulose (rather than zero, which is unlikely). Samyang is requesting the value of 1.0 kJ/g be assigned as the energy factor for D-allulose in section S11—2(3) of the Code.

However, Samyang recognises the human study referenced in the derivation of this FE value is not published in a peer-reviewed format and therefore cannot be independently verified by Samyang or FSANZ. Additionally, other than this study, only animal studies are available in relation to measuring faecal excretion of D-allulose. Samyang considers animal excretion rates are likely to be higher than in humans and therefore not appropriate to use in isolation. Recognising this uncertainty, Samyang has also included an alternative calculation using an FE

value of zero, rather than relying on the unpublished human study and animal studies. If the zero FE value is used, the energy factor for D-allulose would be 1.68 kJ/g, which would be rounded up to 2 kJ/g if listed in section S11—2(3) of the Code. Samyang considers the zero value is not likely to be representative of actual levels of human faecal excretion.

*G.1.1 Substantiation of the proposed energy factor of the food ingredient*

*(addressing section 3.2.5.B.3 of the FSANZ Application Handbook)*

The Application Handbook includes guidelines on the scientific methods that can be used to substantiate the values included in the above equation ((a) to (e) below). Details on how each of the components of the metabolisable energy equation were calculated are included below.

Samyang has calculated an energy factor for D-allulose in accordance with the above formula and using the guidance provided in the Application Handbook. The data and assumptions used by Samyang are summarised below.

| | |
|---|---|
| GE = | 15.2 kJ/g (based on the value for fructose cited by FSANZ in Application A472)<br><br>D-allulose is an epimer of fructose, differing only in the orientation of oxygen and hydrogen atoms at C3. FSANZ's assessment of the novel food, D-tagatose included consideration of an energy factor. FSANZ cited known GE values for glucose and fructose of 15.7 kJ/g and 15.2 kJ/g, respectively in its assessment of a suitable energy factor for D-tagatose[4]. Given the similarities in structure of fructose and D-allulose, Samyang considers it is appropriate to use the 15.2 kJ/g value as GE for D-allulose. |
| FE = | 0 - 0.68 kJ/g (range based on whether assuming zero value or using average of Williamson et al. (2014) values (see below)<br><br>Animal studies have demonstrated D-allulose excretion in faeces ranging from 8-13% (Matsuo et al. 2003, Whistler 1974). A study by Iida et al. (2010) in humans measured urinary excretion and fermentation in the large intestine but did not measure excretion in faeces. Williamson et al. (2014) measured faecal excretion in humans (2-6%). In relation to the Iida et al. (2010) study, the observations about urinary excretion and fermentation of D-allulose are of relevance in considering faecal excretion.<br><br>The study by Iida et al. measured expiratory gas in human subjects after D-allulose consumption and compared this to results for subjects consuming the same quantity of fructooligosaccharide (FOS). Expiratory gas collected after D-allulose consumption was significantly less than after FOS consumption. This is not unexpected, as Iida et al. reported significant excretion of D-allulose in urine, suggesting only a small proportion of D-allulose is likely to pass to the large intestine. The results observed in the Iida et al. study suggest some fermentation of D-allulose by bacteria in the large intestine. Iida et al. also reported limited fermentability of D-allulose by intestinal bacteria strains typically found in humans. Of the 35 bacterial strains tested in-vitro, four exhibited |

[4] See attachment 5 of FSANZ's Final Assessment Report for Application A472: https://www.foodstandards.gov.au/code/applications/Documents/A472_D_tagatose_FAR.pdf

| | |
|---|---|
| | fermentation of D-allulose. This suggests that of the D-allulose that passes to the large intestine, a limited amount is fermented and that which is not fermented must be excreted in faeces. Although the Williamson et al. study is not published, it reported faecal excretion of 2-6% (using $^{14}C$ labelled D-allulose). This is lower than faecal excretion of D-allulose reported in animal studies by Matsuo et al. (2003) and Whistler (1974). Given the higher rates of urinary excretion reported in humans (Iida et al. (2010)) than animals, the rate of faecal excretion in humans is likely to be lower than in animals.<br><br>In the absence of peer-reviewed, published human data on faecal excretion of D-allulose, a conservative estimate such as the average rate of excretion from Williamson et al. is more representative than using animal excretion data, which would result in approximately double the value for FE. An average of the values reported by Williamson et al. is 4%. This 4% value appears to be realistic in the context of the results reported by Iida et al., which suggests some D-allulose must be excreted in faeces due to limited fermentation of D-allulose that reaches the large intestine. Using this 4% value results in an estimated FE of 0.68 kJ/g. Samyang considers this is appropriate for the purposes of this calculation.<br><br>However, given the Williamson et al. (2014) study (which reports on the results of a clinical trial) has not been published, it is not possible to independently assess the data presented in the abstract. If the Williamson et al. data is not used, there is a lack of human data to rely upon in the calculation. Animal data in isolation, as noted above, is likely to be an overestimate of human faecal excretion of D-allulose. Given this uncertainty, Samyang has used two values for FE, depending on whether the Williamson et al. study values are used or not. The Williamson et al. average value of 4% faecal excretion results in an FE value of 0.68 kJ/g. An assumed FE value of zero has also been included in an alternate calculation that does not rely on the values reported in the Williamson et al. study or animal studies.<br><br>Samyang considers the 0.68 kJ/g FE value is the most appropriate value to be used in the calculation of an energy factor using the equation set out in the FSANZ Application Handbook. |
| UE = | 12.67 kJ/g (based on average reported by Iida et al. (2010))<br><br>Iida et al. (2010) reported high urinary excretion of D-allulose after oral administration at three dose levels. Urine was collected for 48 hours after administration of D-allulose in 14 healthy adult subjects. D-allulose in urine was assayed by high-performance liquid chromatography. Cumulative 48-hour recovery of D-allulose in urine for the three dose levels was:<br><br>66.2% ± 12.6% for 0.33 g/kg bw dose;<br>78.6% ± 10.6% for 0.17 g/kg bw; and<br>78.8% ± 11.7% for 0.08 g/kg bw.<br><br>The average of these three values is 74.5%. Although unpublished, the values reported by Williamson et al. are similar to this rate of urinary excretion. However, the Williamson et al. results are not used for this calculation. Using |

| | |
|---|---|
| | the average value of urinary excretion in the Iida et al. (2010) study results in a UE value of 12.67 kJ/g.<br><br>Animal studies have identified lower rates of urinary excretion than in humans. Tsukamoto (2014), using radio labelled D-allulose, reported 33% excreted in urine of rats 2 hours after administration of 100 mg/kg bw. Intravenous administration of the same dose saw 50% excreted via urine within 1 hour. Whistler (1974), using radio labelled D-allulose in rats, reported 35% of an oral dose of D-allulose was excreted in urine after 72 hours (98% for intravenous dose). Matsuo et al. (2003) reported 11-15% urinary excretion 24-hours after an oral dose of 5 g/kg bw in rats. Given the human study published by Iida et al. (2010) is more representative of the fate of orally administered D-allulose in humans, the average urinary excretion rates reported in this study have been used to calculate an estimated value for UE for D-allulose. Although unpublished, the Williamson et al. (2014) study results also indicate a greater urinary excretion rate for D-allulose in humans than in animals. |
| GaE = | 0.85 (based on the default value in the Application Handbook for ingredients fermented or partly fermented in the large intestine)<br><br>Iida et al. (2010) reported low rates of breath hydrogen gas excretion after oral administration of D-allulose. The results for D-allulose were significantly less than rates of breath hydrogen gas excretion after administration of equal doses of FOS. FOS is recognised as a substance that is fermented by microflora in the large intestine. The low rates of breath hydrogen gas excretion measured after D-allulose ingestion suggest that D-allulose is fermented in the large intestine at a much lower rate than FOS. This is supported by the results of an in-vitro study also reported in Iida et al. (2010), finding that only 4 of 35 typical intestinal bacteria showed evidence of fermenting D-allulose.<br><br>Matsuo et al. (2003) reported short chain fatty acid (SCFA) production in the large intestine after D-allulose oral administration in rats. Matsuo et al. (2003) reported increasing cecal weight and surface area with increasing dosage of D-allulose, which is correlated with SCFA production. The authors noted that consumption of non-digestible carbohydrates is often associated with an increased cecal weight (which is not a toxicological concern).<br><br>The Iida et al. study suggests there is a limited amount of fermentation of D-allulose in the large intestine of humans but does not provide a measure of the percentage of D-allulose fermented. Given the lack of reporting of a direct measurement of a percentage of D-allulose that is fermented in the large intestine, the default value of 5% provided in the Application Handbook has been used to provide an estimate of GaE, which results in a value of 0.85 kJ/g. |
| SE = | 0 (based on the default value in the Application Handbook) |

## *G.1.2 Information on other factors that affect the calculation of the energy factor*

*(addressing section 3.2.5.B.4 of the FSANZ Application Handbook)*

### *G.1.2.1 Other studies*

Although not reported in the calculation of the energy factor for D-allulose, some studies provide supporting data for a very low availability of energy following D-allulose ingestion.

Iida et al. (2010) reported results of studies measuring carbohydrate energy expenditure (CEE) in humans associated with D-allulose oral administration compared to starch hydrolysate (both administered at 0.35 mg/kg bw). Respiratory exchange measured for 180 minutes showed CEE increased after starch hydrolysate administration, but no increase was observed after D-allulose administration, nor for administration of water (as expected). This indicates D-allulose is not metabolised into energy at this dose (equivalent to approximately 20 g for a 60 kg individual).

Iida et al. (2010) also assessed fermentability of D-allulose in the large intestine via breath hydrogen analysis for the purpose of estimating an energy value for D-allulose. Breath hydrogen was analysed after oral administration of D-allulose at three dose levels (approximately 5, 10 and 20 g) and compared to results of fructose administration at the same dose levels. Fructose was used as a control because it is known to be fermented in the large intestine and has a recognised energy value of 8 kJ/g. D-allulose breath hydrogen values were significantly lower than fructose values, indicating there was only minor fermentation of D-allulose in the large intestine. Iida et al. estimated an energy value for D-allulose, based on comparison with fructose values, of no more than 1.6 kJ/g for the 5 g dose, decreasing to 0.8 and 0.9 kJ/g for the 10 and 15 g doses, respectively.

In addition, Iida et al. (2010) investigated the potential for habituation/adaptation to consumption of D-allulose. Eight human subjects ingested 5 g of D-allulose three times per day for 8 weeks. End-expiratory gas collection was conducted on the first and last day of the ingestion period (and before D-allulose ingestion began). No significant difference in breath hydrogen excretion after D-allulose ingestion was observed before and after the 8-week adaptation period, suggesting that no adaptation occurred after 8 weeks of ingestion of D-allulose.

Iida et al. (2008) reported oral administration of a 7.5 g dose of D-allulose had no influence on blood glucose concentration in humans. Maeng et al. (2019) reported that D-allulose is stable in human and rat hepatocytes for up to 240 minutes, suggesting that D-allulose is subject to minimal metabolism in the liver. Fructose was significantly matabolised in human and rat hepatocytes during the 240-minute time period, which was expected, given that fructose is known to be metabolised in the liver).

Matsuo et al. (2002a) observed the available energy of D-allulose compared to sucrose and fructose in rats after 20 days of daily feeding. Body weight gain was measured, and body energy gain estimated. Body weight gain and body energy gain increased with increasing administration of sucrose and fructose, but not with D-allulose. D-allulose produced negligible net energy gain (0.007 kcal/g, 0.03 kJ/g) while sucrose and fructose produced net energy gains of 2.29 kcal/g (9.6 kJ/g) and 1.76 kcal/g (7.4 kJ/g) respectively. The authors suggested that D-allulose provides essentially zero energy after oral consumption.

Matsuo et al. (2003) reported that high doses of D-allulose in rats may increase fermentation of D-allulose, observing increased cecal short chain fatty acid levels and cecal weight with increasing doses. This suggests potential for some fermentation of D-allulose in the large intestine for D-allulose that is not excreted in urine.

## G.2 Information to support a change to nutrition information labelling of D-allulose

### *G.2.1 How a new energy factor for D-allulose will change nutrition information labelling*

*(addressing section 3.2.5.A.1 of the FSANZ Application Handbook)*

Assigning D-allulose an energy factor in S11—2(3) of the Code will affect the labelling of nutrition content for foods containing D-allulose as an ingredient. The most obvious impact is the energy content of foods of foods containing D-allulose. However, other impacts include the declaration of carbohydrate and content claims about sugar. Each of these impacts are discussed below.

#### *G.2.1.1 Energy content of foods containing D-allulose*

The average energy content of foods must be calculated in accordance with section S11—2 of the Code. If D-allulose is not assigned a distinct energy factor, the labels of foods containing D-allulose will be forced to reflect the energy factor for carbohydrates listed in S11—2(2), which is 17 kJ/g. As described in section G.1, D-allulose has a much lower energy factor of 1 kJ/g. Therefore, without an energy factor for D-allulose in S11—2(3) of the Code, foods containing D-allulose would be inaccurately labelled as containing significantly more energy than is the case.

The inclusion of an energy factor for D-allulose in section S11—2(3) of the Code will enable food manufacturers using D-allulose as an ingredient in foods to accurately reflect the average energy content of these foods.

#### *G.2.1.2 Declaration of carbohydrate and sugars content*

The carbohydrate content declared in a nutrition information panel can be calculated as ‘available carbohydrate’ or as ‘available carbohydrate by difference’, in accordance with S11—3 of the Code. The use of ‘available carbohydrate by difference provides a simple mechanism to account for carbohydrate ingredients, such as D-allulose, that do not have the typical energy factor of most carbohydrates. ‘Available carbohydrate by difference’ is calculated by subtracting from 100 the average quantity in the food, expressed as a percentage, of the following substances:

(a) water;
(b) protein;
(c) fat;
(d) dietary fibre;
(e) ash;
(f) alcohol;
(g) if quantified or added to the food—any other unavailable carbohydrate;
(h) a substance listed in S11—2(3).

Samyang considers the inclusion of an energy factor for D-allulose in section S11—2(3) of the Code will enable food manufacturers to use the 'available carbohydrate by difference' calculation when determining the correct declaration of carbohydrate content in nutrition information panels of foods containing D-allulose. The content of D-allulose can be subtracted in the above calculation, which means that D-allulose is, appropriately, not required to be included in the calculation of carbohydrate for the purposes of declaring carbohydrate content in the nutrition information panel.

In accordance with section 1.2.8—6(9) of the Code, if D-allulose is listed in section S11—2(3) and is present at no less than 5 g/100 g; and 'available carbohydrate by difference' is used, the nutrition information panel must include individual declaration of D-allulose. That is, D-allulose will need to be listed and quantified in the nutrition information panel if the above conditions are met.

In addition to the carbohydrate content being declared in the nutrition information panel, sugars must also be declared (section 1.2.8—6(1)(d)(ii)(A) of the Code). Sugars are declared as a subset of carbohydrates in nutrition information panels (as indicated by the format of nutrition information panels described in Schedule 12 of the Code). When using 'available carbohydrate by difference' to calculate carbohydrate content of foods containing D-allulose, as described above, the inclusion of an energy factor for D-allulose in section S11—2(3) of the Code will ensure that D-allulose is, appropriately, not included in the declaration of carbohydrate content for the purposes of the nutrition information panel of food labels. Samyang interprets this to indicate that because D-allulose will not be included in the declaration of carbohydrate content, D-allulose should also not be included in the declaration of sugars content for the purposes of the nutrition information panel.

D-allulose has been considered by the US FDA in the context of nutrition labelling. The US FDA released guidance for industry relating to the declaration of allulose and calories from allulose on nutrition and supplement fact labels in October 2020 (FDA 2020). The FDA released the guidance in response to petitions from industry requesting exemptions for allulose from the declaration of 'total carbohydrate', 'total sugars' and 'added sugars' on nutrition facts labels. Although the guidance is not a legal decision, the FDA has advised that it will take a discretionary approach to enforcement of some aspects of labelling associated with D-allulose.

In particular, the FDA noted a discretionary approach will be taken in relation to the exclusion of D-allulose from the declaration of 'total sugars' and 'added sugars. That is, if D-allulose is not included in the declaration of 'total sugars' and/or 'added sugars' on a food label, the FDA is unlikely to take enforcement action. The FDA based this on D-allulose being virtually unmetabolised in the human body, its much lower energy content, and its lack of promotion of dental caries, as distinct from other sugars. However, the FDA considers D-allulose should be declared as part of 'total carbohydrate'. This is due to the fact that the US definition of 'total carbohydrate' is based purely on chemical definition, rather than on physiological effect. Therefore, components such as D-allulose and dietary fibre are included in the 'total carbohydrate' value in the US whilst available carbohydrates are included in the Australian and New Zealand context.

## G.3 Nutrition content claims

The use of D-allulose as a low energy, replacement ingredient for conventional sugars will be attractive to food manufacturers wishing to sell foods with reduced energy and lower levels of energy-laden added sugars. Food manufacturers may wish to make claims about the energy and/or sugar content of foods containing added D-allulose as an ingredient. Standard 1.2.7 of the Code sets out requirements for nutrition content claims. Section 1.2.7—12 includes requirements for nutrition content claims about properties of food in the table to section S4—3. The table to section S4—3 includes properties of food such as energy and sugars, which Samyang considers are the most likely properties to be referenced in the context of nutrition content claims on foods containing D-allulose as an ingredient.

Descriptors of energy content, such as 'low' or 'reduced' are subject to clear requirements in the table to section S4—3 and food manufacturers will need to follow these requirements to ensure any nutrition content claims are compliant. For example, for a 'reduced energy' claim, the food must contain at least 25% less energy than in the same amount of reference food. The inclusion of an energy factor for D-allulose in section S11—2(3) will assist food manufacturers in determining energy content of foods containing D-allulose and therefore in considering whether the conditions for these types of nutrition content claims can be met. Samyang considers the requirements for making nutrition content claims about energy content for foods containing added D-allulose are clear and do not require amendment.

However, Samyang considers the unique metabolic properties of D-allulose that distinguish it from conventional sugars should be reflected in the Code's requirements for sugar(s) nutrition content claims. As highlighted in section 3.4 above, Samyang is requesting FSANZ amend the requirements for sugar(s) nutrition content claims to ensure that foods containing added D-allulose can carry claims such as 'low' or 'reduced' sugar and 'no added sugars' (assuming the content of conventional sugars meets the existing requirements for sugars nutrition content claims). The Code's requirements and more detail on Samyang's request for amendment is included below in section G.3.1. Additional detail on addressing the Application Handbook's requirements for amendments to nutrition content claims requirements is included in section G.3.2.

### *G.3.1 Claims about sugar content for foods containing added D-allulose*

Sugars are defined, for labelling purposes, in section 1.1.2—2 of the Code as:

> ***sugars***:
>
> (a) in Standard 1.2.7, Standard 1.2.8 and Schedule 4 (except where it appears with an asterisk as 'sugars*')—means monosaccharides and disaccharides; and
>
> (b) otherwise—means any of the following products, derived from any source:
>
> (i) hexose monosaccharides and disaccharides, including dextrose, fructose, sucrose and lactose;
>
> (ii) starch hydrolysate;
>
> (iii) glucose syrups, maltodextrin and similar products;
>
> (iv) products derived at a sugar refinery, including brown sugar and molasses;
>
> (v) icing sugar;
>
> (vi) invert sugar;
>
> (vii) fruit sugar syrup;
>
> but does not include:

(i) malt or malt extracts; or

(ii) sorbitol, mannitol, glycerol, xylitol, polydextrose, isomalt, maltitol, maltitol syrup, erythritol or lactitol.

There are two parts to the definition, (a) and (b). In general, for the purposes of Standard 1.2.7, Standard 1.2.8 and Schedule 4, sugars are defined as monosaccharides and disaccharides (part (a)). In the context of Schedule 4 requirements for nutrition content claims about '% free', 'low' and 'reduced' or 'light/lite' sugar or sugars, this definition applies. However, in the context of 'no added sugars' content claims, Schedule 4 includes the term 'sugars*' (with an asterisk). It is Samyang's understanding that in the context of making a 'no added sugars' content claim, part (b) of the sugars definition applies.

D-allulose appears to be subject to both part (a) and part (b) of the definition of sugars because D-allulose is a monosaccharide (part (a)) and a hexose monosaccharide (part (b)). Therefore, under the current Code requirements outlined above, it appears that D-allulose added to foods will need to be included in the consideration of sugars content for the purposes of making 'low', 'reduced', 'light/lite' or '% free' sugar content claims. Similarly, added D-allulose will also need to be included as a sugar in the consideration of 'no added sugars' claims. Based on the above, it appears foods containing added D-allulose, as an alternative to conventional sugars (such as sucrose), will not be able to carry claims relating to reduced sugars content or no added sugars (assuming no other sugars are added).

Samyang considers that foods containing added D-allulose should be permitted to make claims about '% free', 'low', 'reduced', 'light/lite' and 'no added' sugar(s), if the conditions for conventional sugars in the table to section S4—3 are met. That is, Samyang considers D-allulose should be excluded from the conditions for conventional sugars in the table to section S4—3. Samyang is therefore requesting that FSANZ investigate a mechanism in the Code to enable foods containing added D-allulose to carry claims relating to sugar content in the table to section S4—3, if the content of conventional sugars satisfies the existing conditions. Samyang considers this request is justified because of the very low metabolisable energy of D-allulose and in the context of public health concerns about added sugars.

Sugars, particularly added sugars, are associated with excess energy intake, which can lead to overweight and obesity and other adverse health outcomes. Dietary guidelines encourage limiting intake of foods and drinks containing added sugars, based largely on the potential for excess energy intake (NHMRC 2013). As described in section G.1, D-allulose contributes significantly less energy than other sugars. The replacement of added sugars such as sucrose with D-allulose will significantly reduce the energy content of foods and may address, in some part, the public health concern of excess energy intake from added sugars.

The US FDA's guidance for industry on the declaration of allulose on food and supplement labels supports Samyang's request to remove D-allulose from consideration as a sugar for the purposes of nutrition content claims described above (FDA 2020). The FDA's guidance states the FDA will take a discretionary approach to the exclusion of allulose from the 'Total Sugars' and 'Added Sugars' declarations on Nutrition and Supplement Facts labels. This discretionary approach will be taken pending future rule changes that will presumably make this a permanent approach. The FDA noted in its guidance that allulose is virtually unmetabolised in the human body, has a very low caloric value, is not cariogenic and has a negligible effect on glycaemic and insulinemic response.

Samyang is focussed on marketing the low energy and technical properties of D-allulose in Australia and New Zealand. However, recognising the US FDA's guidance addresses potential effects of D-allulose on dental caries and glycaemic and insulinemic response, Samyang has provided commentary below on these issues.

Added sugars in the diet is also a public health concern in relation to the incidence of dental caries (NHMRC 2013). Samyang notes the US FDA's guidance addressed the cariogenic potential of D-allulose. The FDA guidance highlighted that in two *in-vivo* studies, dental plaque pH was reduced less after rinsing with a D-allulose solution than a comparable sucrose solution in both studies. Reduced dental plaque pH is associated with increased incidence of dental caries. A 2013 US patent (Iida et al.) was also discussed in the FDA guidance. The FDA noted the patent identified an *in-vitro* study that inoculated growth media containing various sugars (and a control without sugar) with *Streptococcus mutans*, the bacterium associated with the development of dental caries. The culture medium containing D-allulose showed a higher pH and low bacterial growth compared to glucose, fructose and sucrose containing media; and was similar to the control medium that contained no sugar. However, these studies do not appear to be published in a peer-reviewed format and Samyang has not identified published studies to support these findings relating to D-allulose. Therefore, although D-allulose may not be cariogenic, Samyang considers additional research is required to investigate this potential benefit. Samyang is focussed on marketing the low energy properties of D-allulose in Australia and New Zealand.

The US FDA's guidance also addressed D-allulose's negligible effect on glycaemic and insulinemic responses. Section C.1.2.6.2 of this application also addresses the safety of D-allulose in the context of effects on glucose metabolism. Samyang is focussed on marketing the low energy properties of D-allulose in Australia and New Zealand The US FDA's guidance cited five short-term human studies, two of which were unpublished. The FDA noted the unpublished studies showed statistically significant lower blood glucose concentrations after consumption of 25 g of allulose compared to the same amount of glucose in a water solution. One of the studies was reported to have also measured insulin concentrations, with the AU after ingestion of allulose statistically significantly lower than after glucose ingestion. However, Samyang has not viewed the results of these studies as they are not published. The remaining three studies are also short-term studies and are summarised in section C.1.2.6.2 above, which also includes longer term human studies.

A brief summary of the short-term studies is provided below (see section C.1.2.6.2 for additional detail). Iida et al. (2008) observed D-allulose suppressed elevation of blood glucose concentration when consumed with a maltodextrin solution in healthy adult subjects. D-allulose ingestion alone (without maltodextrin) had no effect on blood glucose or insulin concentrations. Hayashi et al. (2010) observed reductions in blood glucose in adult subjects (some of which were borderline diabetic) 30 and 60 minutes after consuming 5 g of D-allulose in tea with a test meal when compared to control (0 g D-allulose in tea with test meal). The observed effect was greater in borderline diabetic subjects. Noronha et al. (2018) observed modest reductions in postprandial blood glucose response to oral glucose in adult subjects with type 2 diabetes who ingested allulose with the glucose test solution. No reductions were observed in subjects consuming fructose with the glucose test solution. Braunstein et al. (2018) did not identify any effects of small doses of allulose or fructose on postprandial glycaemic responses to an oral glucose tolerance test in healthy adult subjects.

Longer term human studies have also investigated glucose metabolism after D-allulose ingestion. Tanaka et al. (2020) focussed on lipid metabolism parameters over a 48-week period of D-allulose ingestion. However, an oral glucose tolerance test was conducted on the first day and 48 weeks after starting consumption. No significant changes in glucose area under the curve (AUC) were observed overall, but in the sub-group with borderline diabetes the D-allulose group had significantly decreased differential values of glucose AUC compared to placebo. Two 12-week studies (Han et al. (2018b) and Hayashi et al. (2010)) reported no significant differences in fasting blood glucose and insulin values between D-allulose ingesting and placebo groups. No adverse effects from D-allulose ingestion were observed in any of these short- or longer-term studies.

Samyang considers additional research may also be required to substantiate a positive effect of D-allulose consumption on glycaemic and insulinemic response, particularly in borderline diabetic or diabetic consumers. Samyang is therefore not focussing on marketing these potential effects to food manufacturers in Australia and New Zealand. The Code's requirements for health claims ensures that food manufacturers will need to independently substantiate or refrain from making claims of this nature in future.

*G.3.2 Application Handbook requirements for nutrition content claim amendments*

Section 3.2.6.A.1 of the FSANZ Application Handbook includes requirements for applications requesting amendments to Standard 1.2.7 or Schedule 4. Rather than suggest specific amendments to sections of the Code, Samyang is requesting FSANZ investigate the most appropriate amendment(s) to address this application's request to exclude D-allulose from consideration as a sugar for the purposes of making sugar nutrition content claims that are included in section S4—3. Without knowing the exact nature of potential amendments that FSANZ may propose, Samyang considers addressing the section 3.2.6.A.1 Application Handbook requirements is appropriate for this application.

Samyang is an ingredient manufacturer and will be supplying D-allulose to food manufacturers in Australia and New Zealand. The intended uses of D-allulose listed Table D.1-1 of this application are therefore uses that Samyang considers likely. However, food manufacturers may need to develop formulations for food products that substitute conventional sugars with D-allulose to ensure optimal taste and technological profiles. Therefore, it will be up to food manufacturers to determine the levels of addition of D-allulose that best suit their food products.

Samyang recognises that not all formulations of food products containing added D-allulose will satisfy the conditions for conventional sugar content listed in the table to section S4—3 for sugar nutrition content claims. However, where added conventional sugar content is reduced sufficiently, or replaced fully, by D-allulose addition (alone or in combination with intense sweeteners or other non-sugar ingredients), Samyang considers that nutrition sugar content claims such as 'low', 'reduced', 'light/lite' and 'no added' should be permitted.

D-allulose is intended to be used in a variety of food matrices as a replacement for conventional sugar ingredients (such as sucrose). The intended uses are listed in Table D.1-1. Unlike health claims, nutrition content claims are not restricted by the Code to foods of a certain nutritional profile. However, nutrition content claims do have conditions that foods must meet in order to carry a claim about each property of food listed in the table to section S4—3 of the Code. The conditions for sugar nutrition content claims for each type of claim are reproduced below in Table G.3.2-1 (columns 1 and 2). Column 3 of Table G.3.2-1 includes commentary on each of these conditions, including whether any of these conditions are achievable or suitable for target

foods of particular nutrient composition. The commentary assumes that D-allulose is either excluded from the labelling definition of sugars or is not subject to the conditions for sugar nutrition content claims in section S4—3 of the Code.

Table G.3.2-1. Commentary on sugar nutrition content claim conditions

| Nature of content claim | Condition | Comment |
|---|---|---|
| Low (and % Free) | The food contains no more sugars than:<br>(a) 2.5 g/100 mL for liquid food; or<br>(b) 5 g/100 g for solid food | Foods containing ingredients such as fruit or other ingredients with intrinsic sugars, may be less likely to meet this condition, even if added sucrose[#] is replaced or partially replaced by D-allulose. For example, fruit filled pastries are likely to contain intrinsic sugar at more than the 5 g/100 g level and would not meet this condition regardless of how much sucrose is replaced with D-allulose.<br><br>However, foods that contain low levels of intrinsic sugars may meet these conditions if added sucrose is replaced with D-allulose, assuming the total level of sugars (other than D-allulose) meet the conditions in column 2 (left). |
| Reduced or Light/Lite | The food contains at least 25% less sugars than in the same amount of reference food | It is likely that this condition can be met where D-allulose is used to partially replace sucrose in most of the food categories listed in Table D.1-1. D-allulose is likely to replace enough sucrose in food products to ensure that sucrose content is at least 25% less than in the same amount of reference food. For example, if a pastry product contains 20% sucrose, replacement of 5 g of sucrose per 100 g of product would satisfy this condition. Noting that D-allulose is approximately 70% as sweet as sucrose, the level of D-allulose required to replace 5 g of sucrose may be slightly greater to maintain the same taste profile; or may be lowered by concurrent addition of alternative sweeteners, particularly intense sweeteners (where permitted by Schedule 15 of the Code). |
| No added | (a) The food contains no added sugars*, honey, malt, or malt extracts; and<br>(b) the food contains no added concentrated fruit juice or deionised fruit juice, unless the food is any of the following… | 'No added sugars' claims could only be made if D-allulose is the only sugar* that is added to the food. Noting that D-allulose is approximately 70% as sweet as sucrose, this will only be applicable where the taste profile in the final food is suitably achieved by either total replacement with D-allulose or replacement with a combination of D-allulose and other non-sugar ingredients (for example, intense sweeteners, sugar alcohols (where permitted by the Code)). In either case, the only added 'sugars' will be D-allulose, which, as noted above, has minimal metabolisable energy and, in Samyang's opinion, should not be subject to this condition. That is, foods containing added D-allulose should be permitted to carry 'no added sugars' content claims assuming no other sugars listed in the section 1.1.2—2 labelling definition of sugars are added to the food. |

| Nature of content claim | Condition | Comment |
|---|---|---|
| Unsweetened | (a) The food meets the conditions for a nutrition content claim about no added sugar; and<br>(b) the food contains no intense sweeteners, sorbitol, mannitol, glycerol, xylitol, isomalt, maltitol syrup or lactitol | Not applicable. D-allulose provides sweetness and it will not be appropriate to make an 'unsweetened' claim for foods containing added D-allulose |

[#] Sucrose is used in this table as a general descriptor of conventional sugar ingredients

* Defined in part (b) of the labelling definition of sugars in section 1.1.2—2 of the Code

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**Abbreviations**

ALP = alkaline phosphatase
ALT = alanine aminotransferase
AST = aspartate aminotransferase
AUC = area under the curve
bw = body weight
CAMHB = Cation-Adjusted Mueller Hinton Broth
CAMHB-LHB = Cation-Adjusted Mueller Hinton Broth with lysed horse blood
CFU = colony forming unit
CHO-K1 = Chinese hamster ovarian fibroblast
CLSI = Clinical and Laboratory Standards Institute
Codex = Codex Alimentarius Commission
EDI = estimated daily intake
EFSA = European Food Safety Authority
ELISA = enzyme-linked immunosorbent assay
γ-GTP = γ-glutamyl transpeptidase
GMM = genetically modified microorganism
GMP = Good Manufacturing Practices
GRAS = Generally Recognized as Safe
GRN = GRAS notice
HACCP = Hazard Analysis and Critical Control Point
$HbA_{1c}$ = glycosylated haemoglobin
HDL-C = high density lipoprotein-cholesterol
HOMA-IR = homeostasis model assessment as an index of insulin resistance
HPLC = high-performance liquid chromatography
Ig = immunoglobulin
IL = interleukin
IOM = Institute of Medicine
$LD_{50}$ = mean lethal dose
LDL-C = low density lipoprotein-cholesterol
MIC = minimal inhibitory concentration
NHANES = National Health and Nutrition Examination Survey
ND = not detected
NMR = nuclear magnetic resonance
NOAEL = No-Observed-Adverse-Effect-Level
OECD = Organization for Economic Co-operation and Development
OGTT = oral glucose tolerance test
PBS = phosphate-buffered saline
QC = quality control
rRNA = ribosomal RNA
SCFA = short chain fatty acid
SD = Sprague-Dawley
t-PAI-I = total plasminogen activator inhibitor-1
TC = total cholesterol
TG = triglyceride
TNF-α = tumor necrosis factor-α
US FDA = United States Food and Drug Administration
USA = United States of America
VFDB = Virulence Factors Database