Application to amend the Australia New Zealand Food Standards Code Schedule 15 – 'Substances that may be used as food additives' and Schedule 8 – 'Food additive names and code numbers (for statement of ingredients)' to permit the use of luo han guo extract as an intense sweetener

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Part 1

General information

1.1 Applicant details

1.1.1 Contact details

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1.1.2 Nature of business

Saraya Co., Ltd. (hereinafter 'Saraya') is a global company focussed on the development, manufacture and sale of health and hygiene products and services. Saraya has been developing, producing and selling luo han guo extract-sweetened products since 1995 in Japan and other markets, as part of the company's health food product portfolio.

1.1.3 Details of other parties associated with the application

No other companies or organisations were associated with the preparation of this application.

1.2 Purpose of the application

The purpose of this application is to request Food Standards Australia New Zealand (FSANZ) to assess the intense sweetener commonly known as luo han guo extract for approval for use as a food additive. Approval would require an amendment to the Australia New Zealand Food Standards Code Schedule 15 – 'Substances that may be used as food additives' to list the extract as approved for use, and to Schedule 8 – 'Food additive names and code numbers (for statement of ingredients)' to add the extract's prescribed name.

The primary interest held by Saraya is in the export to Australia and New Zealand of tabletop sweetener products containing luo han guo extract and ready-to-consume food products sweetened with luo han guo extract.

1.3 Justification for the application

High levels of consumption of sugar-sweetened foods and beverages has been associated with a range of health problems, including obesity, dental caries and increased risk of developing type 2 diabetes (Australian National Preventive Health Agency, 2014).

As a way of reducing sugar intake, foods and beverages containing intense sweeteners are widely consumed in Australia and New Zealand, especially amongst people with diabetes or impaired glucose tolerance and those on a weight control diet (FSANZ, 2003).

Luo han guo extract exhibits a number of benefits over other already approved intense sweeteners.

Of particular note is the relative lack of bitter taste that is commonly experienced with other sweeteners, especially saccharin and acesulfame K (Kuhn et al., 2004) and steviol glycosides (Kim et al., 2015). This makes luo han guo extract a more palatable intense sweetener to people who are averse to the bitter taste associated with other sweeteners. Other aspects of the sensory profile of luo han guo extract—discussed further in Section 2.1.1—may also be more appealing compared with other intense sweeteners to some consumers.

Secondly, the extract in combination with erythritol makes for a particularly versatile tabletop sweetener, which has high temperature stability (further details in Section 2.3) and thus can be used as a sugar substitute in baking. This is typically not possible with other intense sweeteners due to either a lack of heat stability, as for aspartame, or the introduction of unpleasant aftertastes, as for saccharin (University of Illinois, 2014).

Furthermore, as luo han guo extract is derived from a plant product, it is usually labelled and marketed as a 'natural sweetener'.[1] This appeals to certain consumers for a variety of reasons.

1.3.1 Cost and benefit to the consumer

The benefits listed above are likely to make luo han guo extract-sweetened food products and luo han guo extract-based sweeteners appeal to those

[1] As opposed to 'artificial sweeteners' like aspartame, saccharin, neotame and sucralose.

consumers who are unsatisfied with other sweeteners or are looking for a sugar substitute that can be used in more ways.

Thus, the approval of luo han guo extract as a food additive will likely attract consumers which would otherwise not use intense sweetener-containing products and/or extend the consumer's use of sugar substitute products to new applications such as baking. This would lead to a reduced sugar intake, which would achieve a positive health outcome for the individual consumer.

The cost of luo han guo extract is competitive with other intense sweeteners, so will not pose an increased cost to the consumer compared with alternative products. The increased diversity in intense sweeteners may contribute to greater competition and reduced prices for consumers.

1.3.2 Costs and benefits to industry and business

Approval of luo han guo extract as a food additive will give manufacturers the opportunity to market new products targeting consumers that find appeal in the benefits mentioned above.

The cost of luo han guo extract is competitive with other intense sweeteners, so no additional cost to industry or businesses, including small businesses, is predicted.

1.3.3 Costs and benefits to government

There may be a small cost to state government food safety enforcement agencies in validating the analytical method of analysis for luo han guo extract. Further costs may also be incurred if they choose to analyse for the presence of luo han guo extract more frequently than for existing sweeteners.

There will also be a cost incurred by FSANZ in the assessment of this application and any resulting amendments that are made to the Australia New Zealand Food Standards Code.

1.3.3.1 Impact on international trade

The approval of luo han guo extract as an intense sweetener would bring the Australia New Zealand Food Standards Code in alignment with other countries where the extract is currently approved for use for this purpose, including China, Japan, Canada and the United States (further details in Section 1.8). As China, Japan and the US are the three largest trading partners for Australia (Department of Foreign Affairs and Trade, 2015) and are all within the top four trading partners for New Zealand (New Zealand Treasury, 2015), the alignment of food standards can only serve to strengthen these trade ties.

Trade with China in particular would be increased as luo han guo is currently only grown and harvested on a commercial scale in the southern parts of China.

Saraya is a Japan-based company which is seeking to export luo han guo extract-sweetened products to Australia and New Zealand. Numerous other international businesses could also take advantage of the approval of luo han guo extract to export their own similar products.

1.3.4 Applications made in other countries

Saraya has previously applied to and received approval from Health Canada for the use of luo han guo extract as a food additive in Canada. Further details are given in Section 1.8.

1.4 Information to support the application

Part 2 contains detailed technical information on luo han guo extract. Part 3 contains all currently available information regarding the safety of luo han guo extract for human consumption. Part 4 contains information relating to the expected dietary exposure to luo han guo extract for the Australian and New Zealand populations.

1.4.1 Industry support for the proposed changes to the Code

Approval of luo han guo extract for use as an intense sweetener is of evident interest to the food industry in Australia and New Zealand for the following reasons:

- Demand exists for such products, as demonstrated by the existence in Australia of a luo han guo extract-based tabletop sweetener and sugar replacement product marketed as 'Norbu' by Flujo Holdings Pty Ltd (Norbu Pty Ltd, 2015). This product is sold in Coles supermarkets as of March 2016, despite luo han guo extract not yet being approved for use as a food additive.
- As noted in Section 1.3.2, it will provide manufacturers with the opportunity to market new low joule products that appeal to different target consumers compared with the already approved intense sweeteners.
- A Hamilton, New Zealand-based subsidiary of Guilin GFS Monk Fruit Corp, formerly known as BioVittoria Ltd and now known as Monk Fruit Corp. (2015), is a large manufacturer of intense sweeteners and products employing intense sweeteners, particularly luo han guo extract. The ability to sell their product in their home market would be of obvious interest and benefit to them.

1.4.2 FSANZ data reporting requirements

This section provides details of the literature searches used for completing this application, as may be required by FSANZ for the purpose of completing a Regulation Impact Statement for the Office of Best Practice Regulation.

Throughout this document, references to 'the scientific literature', or 'literature searches' refer to in-depth searching of the following journal and resource databases: ScienceDirect® (Elsevier), Google Scholar, Google Books, SpringerLink and ResearchGate.

Search criteria used for performing literature searches began at a high level, searching for any of the common names or scientific names of luo han guo using full-text or abstract/title only searches. Date ranges were not specified, searching the full history available in each database. Given the relatively limited number of publications directly relevant to the topic, this was largely effective and further drilling-down of search terms or other search criteria wasn't particularly necessary.

Internal company resources have also been used. Cited internal (unpublished) resources are included as attachments to this application, as with all other cited references.

1.5 FSANZ assessment procedure

Saraya suggests that FSANZ adopt the 'major procedure' (Subdivision F) assessment methodology in assessing this request to amend the Australia New Zealand Food Standards Code.

In reaching this conclusion, Saraya has considered that FSANZ will need to complete a safety and risk assessment of high scientific complexity and potentially establish a reference acceptable daily intake (ADI) ahead of the JECFA (see Section 1.8).

1.6 Confidential information

This application does not contain any information that Saraya considers to be either 'confidential commercial information' or 'other confidential information' as defined in the FSANZ Application Handbook.

1.7 Exclusive capturable commercial benefit

As discussed in Sections 1.3.3.1 and 1.4.1 there are numerous companies that manufacture and sell food products containing luo han guo extract, including New Zealand and Australian based businesses.

These commercial entities are *not* related to Saraya and would *not* require the agreement of Saraya in order to benefit financially from the approval of this application to amend the Australia New Zealand Food Standards Code.

Therefore, the approval of this application will *not* confer to Saraya an exclusive capturable commercial benefit as defined in Section 8 of the *Food Standards Australia New Zealand Act* 1991.

1.8 International and other national standards and regulations

An international standard for luo han guo extract, such as would be defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), does not currently exist.

Table 1.1: Estimated daily intake (EDI) for 90th percentile exposure level to mogroside V assumed in GRAS determinations

Population group	Assumed EDIs, mg/kg bw per day			
	GRN 301[a]	GRN 359	GRN 522	GRN 556
General population	2.4	2.65	1.45	1.48
Diabetic adults	3.2	3.51	1.92	1.97
Healthy children	3.5	3.86	2.12	2.17
Diabetic children	3.2	3.55	1.95	1.99

a Values calculated assuming 35% mogroside V content in raw extract
Note: GRN = GRAS notice number
Sources: USFDA 2010, 2011, 2014, 2015

The JECFA has planned in the past to perform a safety assessment of luo han guo extract for addition to the Codex Alimentarius as a food additive. However, it was removed from the 'priority list of substances proposed for evaluation by the JECFA' between April 2014 and May 2015 (JECFA 2014*a*, 2015*b*) and was neither considered nor evaluated at either the 79th or 80th meetings of the committee (JECFA 2014*b*, 2015*a*).

National standards and regulations exist in the countries discussed in the following sections.

1.8.1 United States

The US Food and Drug Administration (USFDA) has approved four Generally Recognized As Safe (GRAS) determinations for the use of luo han guo extract (approved under the name '*Siraitia grosvenorii* Swingle (Luo Han Guo) fruit extract') as a food additive (USFDA 2010, 2011, 2014, 2015). In all cases the USFDA did not have further questions for the applicants before approval.

According to the US Code of Federal Regulations (CFR) Title 21 §170.30(b), approval of GRAS status is dependent upon the following:

> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and information.

GRAS determinations are carried out by experts in the field of food additive safety, as required by CFR Title 21 §170.30(a).

The estimated daily intakes (EDIs) of mogroside V from luo han guo extract that were considered for each GRAS determination are summarised in Table 1.1.

1.8.2 Canada

Saraya made an application to Health Canada for the use of luo han guo extract as a food additive under the name 'monk fruit extract'. Following a detailed safety assessment by Health Canada's Food Directorate, approval was given effective from 2 December 2013 for use of the extract in tabletop sweeteners (Health Canada, 2013). The 'maximum level of use' was set at 0.8%, calculated as mogroside V concentration in the final product (Health Canada, 2015).

1.8.3 Japan

Food additives in Japan are regulated through the *Food Sanitation Act* 2010.[2] Article 10 of the Act provides an exclusion from these regulations for 'natural flavoring agents and articles that have generally been served for human consumption and that are used as additives'. These are categorised as 'existing food additives', which the Ministry of Health, Labour and Welfare (2015) describes as substances that:

> are permitted for use and distribution in Japan, as exception, without through the designation system as provide by the FSA [Food Sanitation Act] for the reason that they are widely used in Japan and have a long history of consumption by humans. They are referred to as existing food additives and placed on the List of Existing Food Additives. This additive status was created in 1995 when the FSA was revised and all additives (not only chemically synthesized substances but also natural origin) came to be subject to the designation system. [sic]

Luo han guo extract is included on the List of Existing Food Additives under the name 'rakanka extract' (The Japan Food Chemical Research Foundation, 2014). It is therefore exempt from the requirements of new food additives and food additives of non-natural origin and can be used freely in food products without restrictions on use or concentration (MHLW 2015).

For more information on the history of use of luo han guo extract in Japan, see Section 4.3.2.

1.8.4 China

Luo han guo extract is listed under the name 'Luohanfruit tincture [*Siraitia grosvenorii* (Swingle) C. Jeffrey]' for use as a food additive in Chinese National Standard GB 2760-2014 National Food Safety Standard Food Additive Usage Standard.[3]

Its classification is as a 'natural flavoring substance permitted in foods'. This classification does not have any associated restrictions on scope of application or maximum allowable concentration levels.

[2] English translation available from the Japanese Law Translation Database System (Ministry of Justice, 2015)

[3] Unofficial English translation available from USDA Foreign Agricultural Service (2015)

1.9 Statutory declarations

Signed statutory declarations for Australia and New Zealand affirming the truth and accuracy of this application are contained in Appendix C.

1.10 Application checklists

Appendix D contains two completed checklists ('general requirements' and 'food additives') confirming all required information is included with this application.

Part 2

Technical information

2.1 Nature and technological function

The technical function that luo han guo extract fulfils is 'intense sweetener'. That is, according to Schedule 14 of the Australia New Zealand Food Standards Code (section S14—2), a substance which:

> replaces the sweetness normally provided by sugars in foods without contributing significantly to their available energy.

Luo han guo extract was first proposed for use as an intense sweetener by Lee (1975), who identified that water and ethanol extraction of the sweet principle of the luo han guo fruit afforded an intensely sweet substance, approximated to be 150 times the sweetness of sucrose.

Extensive studies in the following decades identified the sweet components of the luo han guo fruit to be a variety of mogrosides (cucurbitane glycosides) of varying sweetness (see Section 2.2.1 for detailed chemical properties).

The overall sweetness of the extract depends on the concentrations of the individual mogrosides present in the extract. Pure mogroside V, the primary component in extracts, exhibits a sweetness of between 250 and 400 times that of sucrose (Hussain et al., 1990; Kim and Kinghorn, 2002).

Depending on the mogroside V concentration, the sweetness of luo han guo extracts are typically 150 to 200 times that of sucrose (Fry, 2012; Marone et al., 2008). Kim et al. (2015) in a study of various intense sweeteners found that luo han guo extract only exhibited a sweetness 75 times that of sucrose. However, the authors noted that the unexpectedly-low result may have been caused by the 'composition of mogrosides in the [extract] or other factors affecting sweetness perception, such as interaction between flavors and dynamics of sweetness progress', acknowledging that the existing literature value of 150 is an accurate relative sweetness for luo han guo extract.

A general nutrient compositional analysis performed for Saraya by the Japan Food Research Laboratories found that the specific available energy of a typical luo han guo extract was 16 kJ/g (Saraya Co Ltd, 2006*a*). Therefore, at the maximum proposed concentration of use of 0.8% (see Section 4.1), luo han

guo extract can provide the sweetness level normally provided by sugars in the listed foods while only contributing around 130 J of energy per gram of food substance.

Therefore it can fulfil the technical function of intense sweetener as defined in Schedule 14.

The need for using luo han guo extract to fulfil the technological function of intense sweetener is discussed in Section 1.3. Specifically, compared to other intense sweeteners, it has a different sensory profile (as discussed below) and is able to be used in baking and cooking in place of sugar.

2.1.1 Sensory profile

Besides the sweetness levels discussed above, the other characteristics of the sensory profile of luo han guo extract are also of great importance to its applicability for use as an intense sweetener.

Many intense sweeteners exhibit an unpleasant bitterness (Kim et al., 2015; Kuhn et al., 2004), which may put people off consuming them with certain foods, perhaps limiting their use of intense sweeteners to applications like adding to coffee where the bitter taste is masked somewhat by the bitterness of the coffee itself.

However, in the sensory evaluation completed by Kim et al. it was found that luo han guo extract had a bitterness roughly equivalent or less than that exhibited by sucrose.

Other characteristics of the sensory profile were found to be a honey odour and flavour, and licorice flavour. Lee (1975) also described a lingering taste accompanying the sweetness as 'licorice-like'.

2.2 Information to enable identification

Luo han guo extract is derived from the fruit of *Siraitia grosvenorii* (Swingle) C. Jeffrey, a vine native to southern China. The first botanical description of the plant was by Swingle (1941), who named it *Momordica grosvenorii*. Its genus has twice been reclassified since, firstly becoming *Thladiantha grosvenorii* (Swingle) C. Jeffrey, and finally then *Siraitia grosvenorii*.

The fruit of the plant has been used whole or in dried powder form for hundreds of years in China and by the Chinese diaspora for the preparation of beverages and traditional medicines (Fry, 2012; Lee, 1975; Swingle, 1941). The fruit itself is known by a number of names, given in Table 2.1 on the following page.

The extract of the fruit is prepared on a commercial scale by the process described in Section 2.5. The extract is known by a number of names, both common and commercial, given in Table 2.2 on the next page. Saraya proposes the use of 'luo han guo extract' as the common name to be adopted by FSANZ for the purpose of regulation, as this is the most common name encountered in the literature relating to the extract, and clearly identifies the source of the extract.

Table 2.1: Common names of the fruit

Language	Common name(s)
English	Luo han guo / luo han kuo
	Lo han guo / lo han kuo
	Monk fruit
	Longevity fruit
	The Buddha's fruit
	Arhat fruit
	Fructus Momordicae
	Siraitiae fructus
Chinese	Luóhàn guǒ
Japanese	Rakanka / lakanka
Vietnamese	La hán quả

Table 2.2: Names of the fruit extract

Context	Name(s)
Common names	Luo han guo extract
	Luo han fruit concentrate
	Monk fruit extract
Within scientific literature	*Siraitia grosvenorii* extract
	Momordica grosvenorii extract
Product brand names	PureLo® (BioVittoria Ltd)

2.2.1 Chemical composition

The sweet components of the luo han guo fruit are cucurbitane triterpene glycosides, known collectively as mogrosides, which make up approximately 1–2% w/w of the fresh fruit (Fry, 2012; Hussain et al., 1990). Early studies of the fruit in 1983 by Takemoto et al. (cited in Makapugay et al. 1985) elucidated the chemical structures of the major component mogrosides, namely mogroside IV and mogroside V, and a minor component, mogroside VI. Numerous studies that followed discovered a wide range of other minor components within the fruit—a total of 37 triterpenoids and 14 flavonoids and related compounds have now been isolated and identified, as summarised by Li et al. (2014).

The primary component of all commercial luo han guo extracts is mogroside V. The concentration of mogroside V is typically 30–40%, but there are preparations of up to 90% mogroside V available (USFDA 2015). A typical composition for the luo han guo extract used by Saraya is given in Table 2.3 on the following page. The structural formula of mogroside V is shown in Figure 2.1 and identification details given in Table 2.4 on page 18. Identification details of the minor components are given in Tables 2.5 through 2.7 and structural formulae shown in Figures 2.2 through 2.4 on the following pages.

Table 2.3: Typical composition of luo han guo extract

Compound	Concentration, %
Mogroside V	30–40
11-oxomogroside V	1–10
Siamenoside I	1–10
Mogroside IV	1–10
Water	0–6
Ash content	0–2
Protein fragments	Balance

2.3 Information on the chemical and physical properties

Typical chemical and physical properties of luo han guo extract are given in Table 2.8 on page 21.

Due to the use of spray drying in the production process (see Section 2.5), the particle size of luo han guo extract is typically 100–250 µm. However, particle size is not significant for the performance of the technological function.

Detailed information regarding metabolic fate is given in Section 3.1.

2.3.1 Stability

Testing performed by Saraya on Lakanto S, a tabletop sweetener containing 0.8% luo han guo extract and 99.2% erythritol, has shown that mogroside V concentration does not diminish during long-term storage at room temperature. The oldest sample tested was stored for a duration of 5 years and the mogroside V concentration remained above the original specification (Saraya Co Ltd, 2006*b*).

Similarly, samples taken from bulk stored pure luo han guo extract with storage durations up to 3.3 years did not show significant change from the originally measured concentration of mogroside V.

The thermal stabilities of both Lakanto S and pure luo han guo extract were also tested. Although the pure extract did exhibit some degradation when heated at 120 °C for 6 hours, Lakanto S did not show significant degradation for the same conditions. The pure extract showed no degradation when heated at 90 °C for 2 hours, which is beyond the conditions that the pure extract is likely to be subjected to.

Lee (1975) also identified by thin layer chromatography that luo han guo extract appeared to be stable in boiling water for 5 hours.

2.4 Information on the impurity profile

Appendix A contains certificates of analysis of four different batches of luo han guo extract for impurities in the following categories.

Table 2.4: Identification details for mogroside V

Identifier	Detail
IUPAC systematic name	(2R,3R,4S,5S,6R)-2-[[(2R,3S,4S,5R,6R)-6-[[(3S,8S,9R,10R,11R,13R,14S,17R)-17-[(2R,5R)-5-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-3-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxy-6-hydroxy-6-methylheptan-2-yl]-11-hydroxy-4,4,9,13,14-pentamethyl-2,3,7,8,10,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-yl]oxy]-3,4,5-trihydroxyoxan-2-yl]methoxy]-6-(hydroxymethyl)oxane-3,4,5-triol
Synonym	Mogrol-3-O-(β-D-glucopyranosyl(1→6)-β-D-glucopyranoside)-24-O-((β-D-glucopyranosyl(1→2))-(β-D-glucopyranosyl(1→6))-β-D-glucopyranoside)
Molecular formula	$C_{60}H_{102}O_{29}$
CAS Registry number	88901-36-4

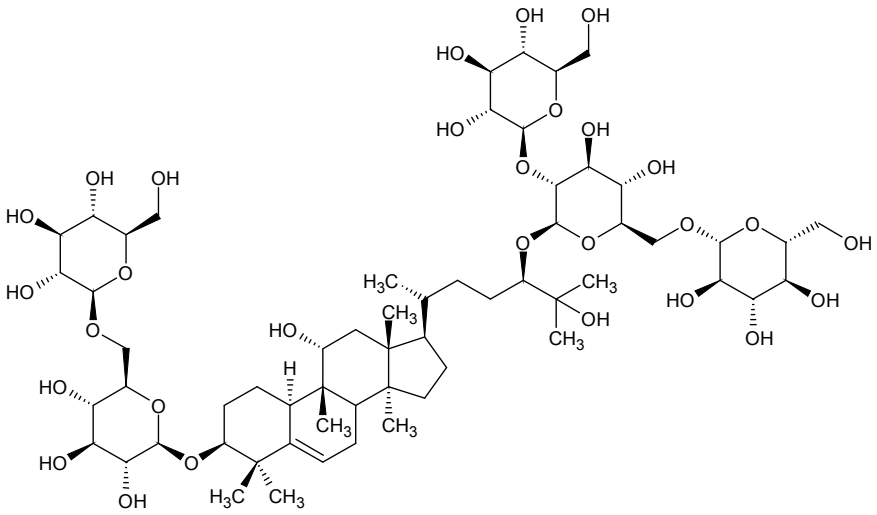

Figure 2.1: Structural formula of mogroside V

Table 2.5: Identification details for 11-oxomogroside V

Identifier	Detail
IUPAC systematic name	(3S,8S,9S,10S,13R,14S,17R)-17-[(2R,5R)-5-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-3-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxy-6-hydroxy-6-methylheptan-2-yl]-4,4,9,13,14-pentamethyl-3-[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl]oxy-1,2,3,7,8,10,12,15,16,17-decahydrocyclopenta[a]phenanthren-11-one
Synonym	Cucurbit-5-en-11-one-3β,24,25-triol-3-O-(β-D-glucopyranosyl(1→6)-β-D-glucopyranoside)-24-O-((β-D-glucopyranosyl(1→2))-(β-D-glucopyranosyl(1→6))-β-D-glucopyranoside)
Molecular formula	$C_{60}H_{100}O_{29}$
CAS Registry number	126105-11-1

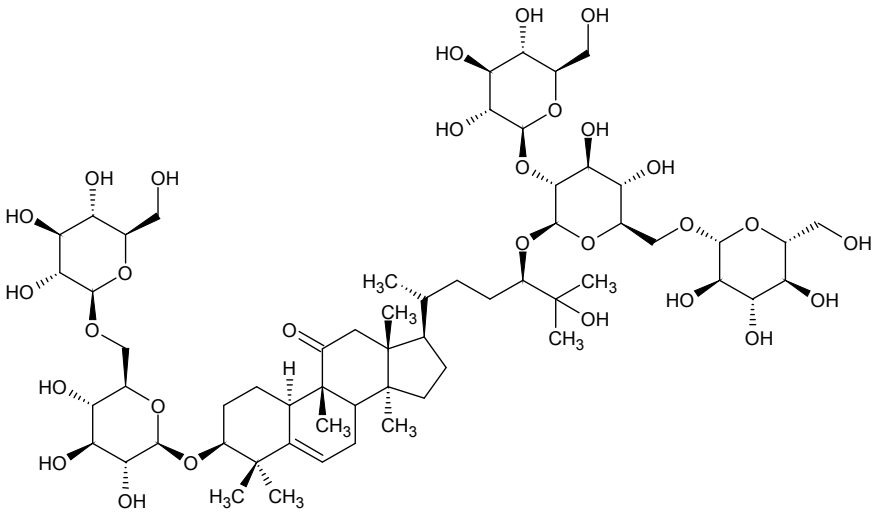

Figure 2.2: Structural formula of 11-oxomogroside V

Table 2.6: Identification details for siamenoside I

Identifier	Detail
IUPAC systematic name	(2R,3R,4S,5S,6R)-2-[[(2R,3S,4S,5R,6S)-3,4-dihydroxy-6-[(3R,6R)-2-hydroxy-6-[(3S,8S,9R,10R,11R,13R,14S,17R)-11-hydroxy-4,4,9,13,14-pentamethyl-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2,3,7,8,10,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-methylheptan-3-yl]oxy-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]methoxy]-6-(hydroxymethyl)oxane-3,4,5-triol
Synonym	Mogrol-3-O-β-D-glucopyranoside-24-O-((β-D-glucopyranosyl(1→2))-(β-D-glucopyranosyl(1→6))-β-D-glucopyranoside)
Molecular formula	$C_{54}H_{92}O_{24}$
CAS Registry number	126105-12-2

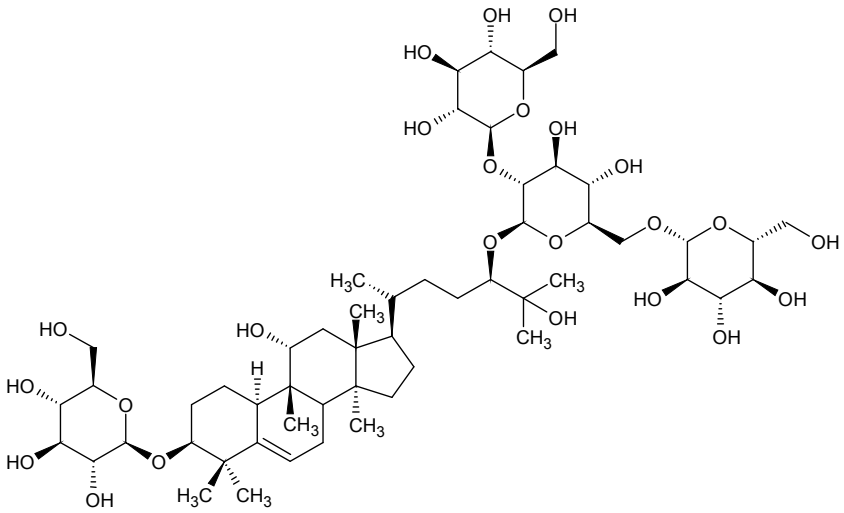

Figure 2.3: Structural formula of siamenoside I

Table 2.7: Identification details for mogroside IV

Identifier	Detail
IUPAC systematic name	(2R,3R,4S,5S,6R)-2-[[(2R,3S,4S,5R,6R)-6-[[(3S,8R,9R,10S,11R,13R,14S,17R)-17-[(5R)-5-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-[(2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-2-yl]oxy-6-hydroxy-6-methylheptan-2-yl]-11-hydroxy-4,4,9,13,14-pentamethyl-2,3,7,8,10,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-yl]oxy]-3,4,5-trihydroxyoxan-2-yl]methoxy]-6-(hydroxymethyl)oxane-3,4,5-triol
Synonym	Mogrol-3-O-(β-D-glucopyranosyl(1→6)-β-D-glucopyranoside)-24-O-((β-D-glucopyranosyl(1→6))-β-D-glucopyranoside)
Molecular formula	$C_{54}H_{92}O_{24}$
CAS Registry number	89590-95-4

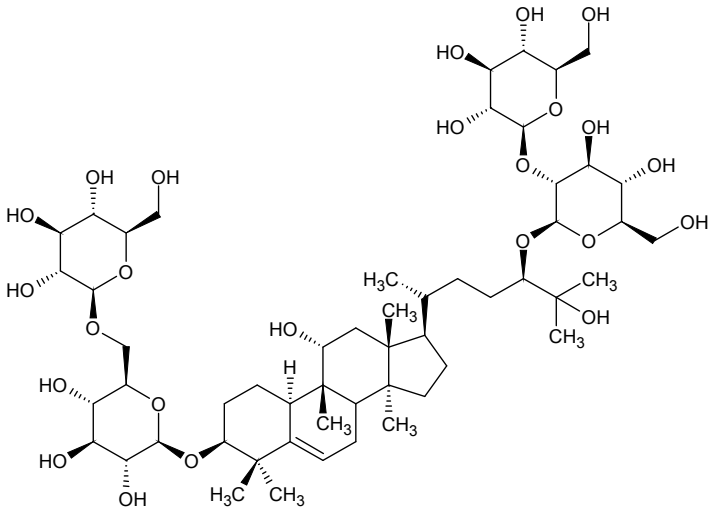

Figure 2.4: Structural formula of mogroside IV

Table 2.8: Chemical and physical properties of luo han guo extract

Property	Description / value
Appearance	Light yellow-brown powder
Odour	Slightly sweet characteristic odour of luo han guo fruit
Melting point	Approximately 310 ºC (depends on exact composition)
Moisture content	less than 6%
Ash content	Less than 2%
Solubility	Readily soluble in water

Inorganic impurities as specified by the United States Pharmacopeial Convention (USP) Food Chemical Codex monograph (USP 2014, pp. 817–818) are given in Table 2.9 on the next page. Total heavy metal content is less than 10 mg/kg.

Biological impurities determined by microbiological analyses are shown in Table 2.10.

Pesticide residues determined by gas chromatography are shown in Table 2.11.

A comprehensive impurity inspection report issued by the Tokyo Metropolitan Institute of Public Health is provided in Appendix B. The analyses performed for this inspection report cover a wider range of analytes than is normally tested for during manufacture, to provide greater assurance of product safety.

2.5 Manufacturing process

The manufacturing process of luo han guo extract is shown as a block flow diagram in Figure 2.5. The major steps are:

- Multi-stage solid-liquid extraction process using water as the solvent to extract mogroside V (amongst other components) from crushed luo han guo fruit. Each of the three stages of extraction last approximately 60 minutes and employ deionised water at 60 °C. The extract solutions from each stage are then combined.
- Precipitation of protein compounds by heating to 100 °C and removal by centrifugation. This results in a clear solution.
- Filtration using ultrafiltration membranes to remove pectin.
- Solid-phase extraction, involving:
  - Adsorption of mogrosides onto a divinylbenzene copolymer resin (D101) column. Unwanted compounds including salts and sugars remain in the mobile phase, which is disposed of as waste.
  - Desorption of the desired compounds from the resin column using a 60% ethanol solution (food grade).
- Distillation to recover ethanol for reuse.
- A second solid-phase extraction utilising a styrene divinylbenzene copolymer resin (LSA-700) column, adsorbing the unwanted non-triterpene glycosides and leaving the desired triterpene glycosides in the mobile phase. This also decolours the solution.
- Vacuum concentration of the solution to approximately 20% solid content. Also removes most of the remaining ethanol.
- Spray drying at 120 °C to form a dry powder and remove the final traces of ethanol and most of the water content.

Good manufacturing practices (GMP) are employed in order to ensure a food-safe product.

Table 2.9: Inorganic impurities in luo han guo extract

Species	Impurity level, mg/kg
Arsenic	<0.5
Cadmium	<1.0
Lead	<1.0

Table 2.10: Biological impurities in luo han guo extract

Analyte	Impurity level
Total plate count	<1000 CFU/g
Yeast and mould	<100 CFU/g
Aflatoxins	<0.2 ppb
Salmonella	Nil
E. coli	Nil
Staphylococcus	Nil

Table 2.11: Pesticide residues in luo han guo extract

Pesticide	IUPAC systematic name	Impurity level, ppm
666 (lindane)	(1r,2R,3S,4r,5R,6S)-1,2,3,4,5,6-hexachlorocyclohexane	<0.1
DDT	1,1′-(2,2,2-trichloroethane-1,1-diyl)bis(4-chlorobenzene)	<0.1
Acephate	N-(methoxy-methylsulfanylphosphoryl) acetamide	<0.1
Methamidophos	O,S-dimethyl phosphoramidothioate	<0.1
Parathion	O,O-diethyl O-(4-nitrophenyl) phosphorothioate	<0.1
PCNB	Pentachloronitrobenzene	<0.01

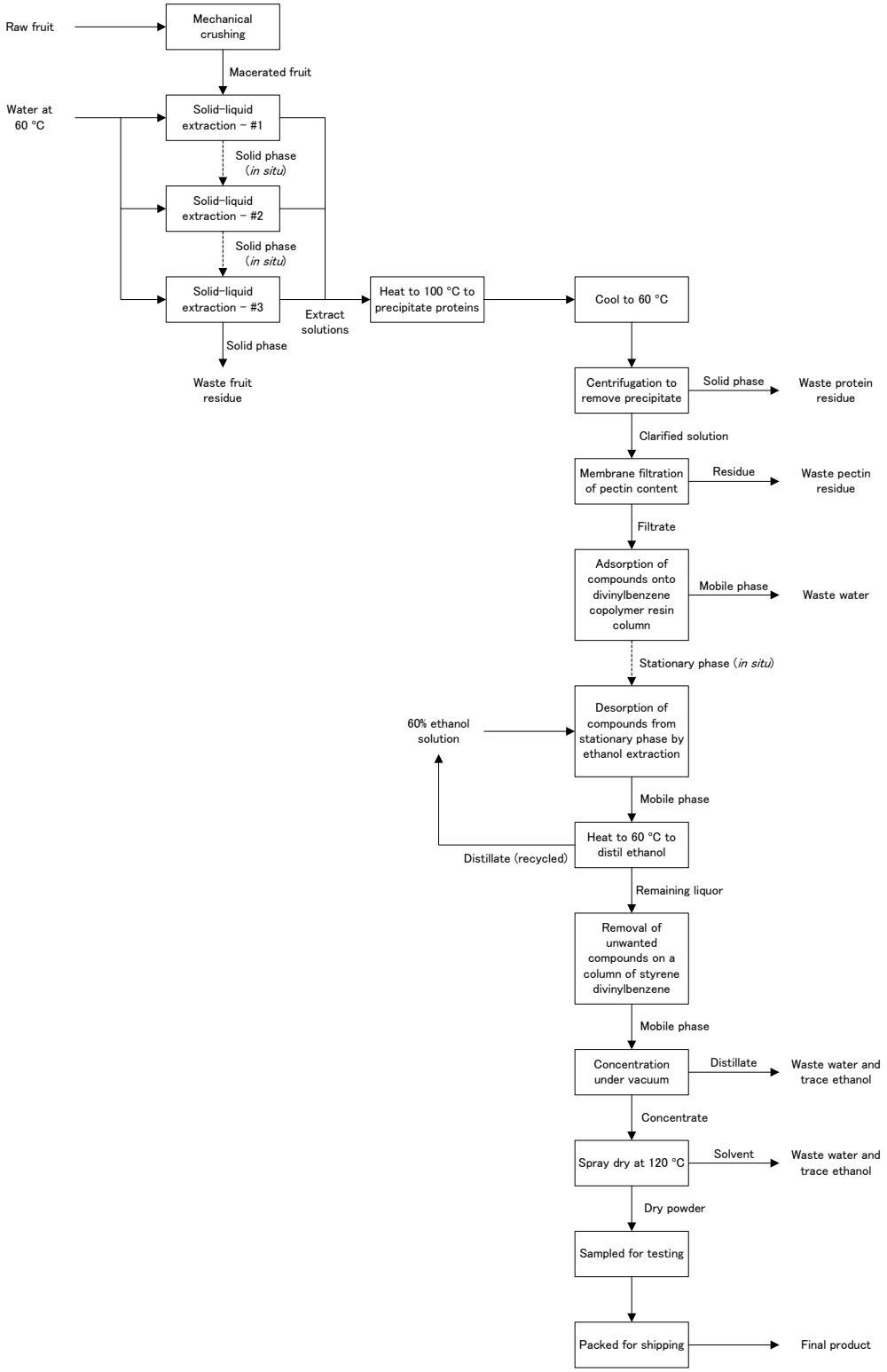

Figure 2.5: Block flow diagram of luo han guo extract production

2.6 Specification for identity and purity

For the purposes of identity and purity specification a monograph for luo han guo extract exists in the United States Pharmacopeial Convention (USP) Food Chemical Codex, listed as 'Monk Fruit Extract' (USP 2014, pp. 817–818). As the USP FCC is listed as a 'primary source' in section 2 of Schedule 3 of the Australia New Zealand Food Standards Code (subsection S3—2(1)), section 1.1.1—15 is satisfied and no amendment to Schedule 3 will be necessary for the specification of identity and purity for luo han guo extract.

There are no known common allergens in commercial preparations of luo han guo extract.

2.7 Information for food labelling

For the purposes of ingredient labelling luo han guo extract is in the functional class of 'sweetener', as per Schedule 7 of the Australia New Zealand Food Standards Code.

As the Codex Alimentarius has not assigned luo han guo extract an INS code number, ingredient labelling would be of the form 'sweetener (*[prescribed name]*)', using the prescribed name assigned by FSANZ. As discussed in Section 2.2, Saraya suggests using the common name 'luo han guo extract' for regulatory purposes.

An amendment to Schedule 8 – 'Food additive names and code numbers (for statement of ingredients)' of the Australia New Zealand Food Standards Code would be required to define the prescribed name for food product labelling purposes.

2.8 Analytical method for detection

Existing Canadian and US regulations relating to the use of luo han guo extract define the maximum concentrations of use in terms of the mogroside V content (see Section 1.8). Detection and quantification of mogroside V in a food matrix is performed using HPLC-UV with an ODS column under the following parameters:

column NUCLEOSIL® 100-5 C_{18} (5 µm particles, 100 Å pores, 15% C, endcapped) 4.6 mm ID, 150 mm length

mobile phase 10% acetonitrile: 90% water

flow rate 1 mL/min

temperature 40 °C

injection volume 20 µL

detection UV 203 nm

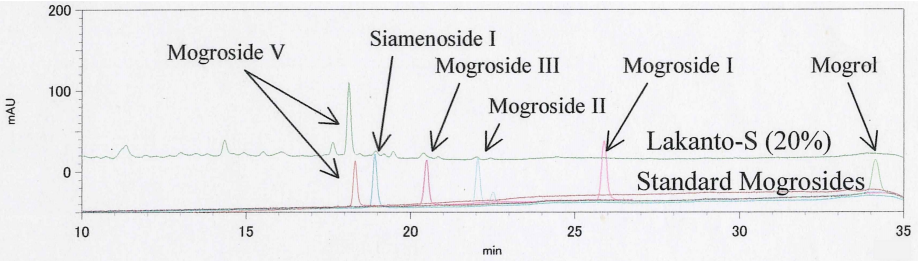

Figure 2.6: HPLC chromatogram of Lakanto S tabletop sweetener

Mogroside V elutes at approximately 18.1 min.

Figure 2.6 shows a compilation of chromatograms obtained using this method for the quantification of mogroside V in a sample of Saraya's tabletop sweetener product, Lakanto S (0.8% luo han guo extract and 99.2% erythritol). The bottom traces are of a variety of mogroside standards, as labelled. The top trace is of a 20% solution of Lakanto S, showing a prominent peak matching that of the mogroside V standard. Quantification of the amount of mogroside V present in the sample is calculated by comparing the chromatogram peak areas of the sample and of a standard of known concentration.

Pure mogroside V standards for analytical use are available from the US Pharmacopeial Convention (catalog number 1445448).

2.9 Potential additional functions when added to food

A wide variety of potential biological activities of *Siraitia grosvenorii* extracts (from various parts of the plant, not just the fruit) have been studied in the past 30 years. A summary of these studies is given in the pharmacological review performed by Li et al. (2014).

The maximum concentration proposed for use as a food additive is 8000 mg/kg in a tabletop sweetener, or 3200 mg/kg of mogroside V (see Section 4.1). Assuming two serves of 3 g each of the tabletop sweetener are added to a 250 mL beverage, this would equate to a final concentration of 77 mg/L of mogroside V. At around this concentration the following biological effects have been observed:

Anti-tussive at doses above 80 mg/kg (98% mogrosides) orally; *in vivo* mouse studies.

Phlegm-expelling at doses above 50 mg/kg (high purity mogrosides) orally; *in vivo* mouse study.

A summary of the studies relating to these two biological activities is included in the review by Li et al. (2014).

Other biological effects have been observed at significantly higher than expected exposures (greater than 100 mg/kg bw) including: liver-protection

and blood glucose regulation functions, and anti-bacterial, anti-carcinogenic, anti-fatigue, anti-inflammatory, anti-allergenic and immunostimulatory effects (Li et al., 2014).

However, it should be noted that evidence for all of these biological activities is limited and not currently supported by human clinical evidence.

Part 3

Food safety information

3.1 Toxicokinetics and metabolism

Two detailed *in vivo* studies of the metabolic fate of mogroside V are reported in the scientific literature: Murata et al. (2010) and Xu et al. (2015).

Murata et al. studied the contents of the small intestine, portal blood, whole blood, faeces and urine of rats that were administered luo han guo extract (72% mogroside V) orally. They found that most of the mogroside V was converted by deglycosylation reactions to mono- and di-glucosides (specifically mogroside IE and mogroside IIA, respectively) and mogrol (the aglycone of the mogrosides), the majority of which was excreted in the faeces—accounting for 61% of the administered dose. The absence of any triterpenoids in the urine or whole blood suggested to the authors that absorption overall of mogroside V or its metabolites was extremely low. The remaining 39% balance of the administered dose was postulated to have been excreted in the faeces as other unknown metabolites. Murata et al. also concluded that mogroside IIA was produced by intestinal microflora, due to the absence of it in the small intestine but large amount present in the faeces.

Xu et al. studied the metabolism of Mogroside V (>98% purity) in three systems: a human intestinal bacteria incubation system (*in vitro*), a rat hepatic 9000 g supernatant (S9) incubation system (*in vitro*), and live rats. Analysis was performed by HPLC-ESI-IT-TOF-MS^n. They identified 77 distinct metabolites, eight of which were identified by comparison with high purity standards, namely: siamenoside I, mogroside IVE, mogroside IIIE, mogroside $IIIA_1$, mogroside IIE, mogroside IIA_2, 11-oxomogroside IIE, and mogrol. These all represent compounds obtained by deglycosylation of mogroside V to varying degrees. The remaining 69 isolated metabolites were identified tentatively by mass spectroscopy data. Four of these were identified as isomers of mogroside VI, indicating a glycosylation reaction. All of the remaining 65 compounds were identified as products of successive hydroxylation, dehydrogenation, methylation or isomerisation reactions on the eight positively identified metabolites above. The majority (46 of them) being mogrol with varying degrees of hydroxylation and dehydrogenation. Xu et al. provide full details of the study including a diagrammatic representation of the

various identified metabolic pathways.

For the *in vivo* rat experiments, they analysed the content of the faeces, urine, blood plasma, heart, liver, spleen, lung, kidney, stomach and intestine. Most of the metabolites accumulated primarily in the faeces with only trace amounts found in the blood plasma, supporting the findings of Murata et al..

However, Xu et al. found significant accumulation of mogroside IIE in the heart, liver, spleen, lung and intestine, which differs from the conclusions made by Murata et al. that very little of the metabolites are absorbed. In fact, the 39% mass deficiency noted above in the Murata et al. study (which was assumed by the authors to be some unknown metabolites) is very close to the proportion of metabolites found by Xu et al. in the organs that were not analysed by Murata et al.. Xu et al. also performed an exhaustive analysis of all isolated components and were able to confidently identify all metabolites, so it is very unlikely that there are additional unknown metabolites.

Xu et al. concluded that seven of the metabolites are bioactive: siamenoside I; mogroside IVE; mogroside IIIE; mogroside IIE; mogroside IA_1; mogroside IE_1; and mogrol. They suggest that it is these metabolites, and especially mogroside IIE, that are responsible for the pharmacological effects that have been observed from high doses of luo han guo extract or of pure mogroside V, as discussed in Section 2.9 of this application. The other metabolites were mainly found in the gastrointestinal tract and faeces only, with little or no uptake to the rest of the body. Mogroside V itself was found throughout all analysed organs (although primarily in the gastrointestinal tract) and the blood plasma, and was expelled in the urine rather than the faeces.

Finally, a brief *in vitro* study of the biotransformation of mogroside III by human intestinal bacteria was reported in Chinese with an English abstract by Yang et al. (2007). They found that that mogroside III underwent deglycosylation to yield mogroside IIA_1 and mogrol, which supports the findings of both Murata et al. and Xu et al. that the metabolites of mogrosides are mostly formed by successive deglycosylation reactions, eventually resulting in mogrol.

3.2 Toxicity data

3.2.1 Acute toxicity

Lee (1975) in his original research into the potential for using luo han guo extract as an intense sweetener performed some preliminary acute toxicity studies in mice. It was found the LD_{50} exceeded 10 g/kg bw, with no mice dying during the study at that dose. Two extracts of luo han guo were tested in this study—a 'crude extract' and a 'treated extract' using a similar method of preparation as is used industrially. Although not stated, the mogroside V content of the treated extract would likely have been 15–25% as only one solid-phase extraction was performed.

Makapugay et al. (1985) reported that acute toxicity experiments in mice resulted in no mortality at doses up to the maximum administered—2 g/kg bw—using pure mogroside V which was prepared as a reference standard.

Shirasu (1990) performed acute toxicity tests in mice for a range of phytochemical compounds including luo han guo extract at doses in the range of 10–2000 mg/kg bw. No mortality was recorded. The concentration of mogroside V in the luo han guo extract used was not stated. However, as a commercial-grade extract, it would be approximately 30–40%.

Hussain et al. (1990) also performed toxicity testing, including for acute toxicity in mice. The doses tested were 1 and 2 g/kg bw. No mortality occurred. Mogroside V concentration is unknown, as it was not stated and the preparation method was different to that normally used for commercial extracts.

No observed adverse effect level

As the study by Makapugay et al. used pure mogroside V for testing, the dosage level of mogroside V administered must exceed that of both the Shirasu and Hussain et al. studies. Therefore, the no observed adverse effect level (NOAEL) for acute toxicity of pure mogroside V is 2 g/kg bw, as determined by the Makapugay et al. study.

Assuming the extract used by Lee had a mogroside V concentration of 20%, this would also equate to a NOAEL for mogroside V of 2 g/kg bw.

3.2.2 Short-term toxicity

Marone et al. (2008) reported the results of a 28-day dietary toxicity study in rats of PureLo® luo han guo extract (39% mogroside V concentration). At doses up to 100 000 ppm, no significant adverse effects or intolerances were observed. This represented a NOAEL for the extract of 7.07 g/kg bw per day for male rats and 7.48 g/kg bw per day female rats. The authors pointed out that the study was 'performed in full conformance with accepted US FDA and OECD guidelines' for toxicity testing of food additives.

No observed adverse effect level

In terms of mogroside V, this would equate to a NOAEL for short-term toxicity of 2.76 g/kg bw per day for male rats and 2.92 g/kg bw per day female rats.

3.2.3 Long-term toxicity

Xiaojian et al. (1996) completed a 90-day study of luo han guo extract (mogroside V concentration unknown) in dogs. The dose administered was 3 g/kg bw per day. The dogs had the following examinations: haematology, blood chemistry, body mass, liver function, renal function, blood and urine sugar, and histopathology. The behaviour of the animals and their food and water consumption and urine and stool excretions were also monitored. No adverse effects were observed in any of these tests.

Qin et al. (2006) completed a very similar 90-day study in dogs given 3 g/kg bw per day of PureLo® luo han guo extract (39% mogroside V

concentration). Examinations completed throughout the study were: clinical observations, body weight, food consumption, haematology, blood chemistry, urinalysis, gross necropsy, organ weight, and histopathology. It was found that the extract was well tolerated and did not produce any toxic effects or significant adverse effects. The authors concluded that the NOAEL for PureLo® luo han guo extract is 3 g/kg bw per day. This equates to a NOAEL for mogroside V of 1.2 g/kg bw per day.

Jin et al. (2007) reports on a 13-week repeated dose toxicity study in rats. The animals were administered a diet including 0–5% luo han guo extract provided by Saraya to the study's authors. Although not stated in the report, the concentration of mogroside V in Saraya's luo han guo extract is typically 31–33%. The following examinations were undertaken throughout the study: general appearance, body weight, food and water consumption, haematological and serum biochemical parameters, organ weight and histopathology. No deaths, adverse effects or toxic effects were observed in any of the animal groups, including the maximum 5% dose which represents a NOAEL for luo han guo extract of 2520 mg/kg bw per day in males and 3200 mg/kg bw per day in females. Conservatively assuming a mogroside V concentration of 30% in the extract, this equates to a NOAEL for mogroside V of 756 mg/kg bw per day in males and 960 mg/kg bw per day in females.

No observed adverse effect level

The highest long-term toxicity NOAEL for luo han guo extract was 3.2 g/kg bw per day. And for mogroside V, 1.2 g/kg bw per day

3.2.4 Genotoxicity

Shirasu (1990), in addition to the acute toxicity tests referred to above, completed *in vivo* mouse bone marrow micronucleus tests using luo han guo extract at three doses: 500, 1000 and 2000 mg/kg bw. The study concluded that all doses tested did not induce micronuclei in the bone marrow of mice. As noted above, as a commercial-grade extract the mogroside V concentration would have been approximately 30–40%.

Jin et al. (2007) in their long-term toxicity study referred to above cite unpublished data from the Japanese Ministry of Health, Labor and Welfare, which confirmed the genotoxicity of luo han guo extract to be negative in an *in vitro* chromosome aberration study and an *in vivo* micronucleus test. Details of the unpublished data are not known.

No observed adverse effect level

The Shirasu study establishes a genotoxicity NOAEL for luo han guo extract as 2 g/kg bw. Conservatively assuming a mogroside V concentration of 30% in the extract used, this would equate to a NOAEL for mogroside V of 600 mg/kg bw

3.2.5 Mutagenicity

Makapugay et al. (1985) briefly mention that results of mutagenicity testing performed in their laboratory showed that pure mogroside V was not mutagenic. However, no other detail is given.

Hussain et al. (1990) report the results of a series of forward mutation assays performed using *Salmonella typhimurium* with and without a 9000 g supernatant (S9) from rat livers. At a variety of doses up to 100 µg, luo han guo extract showed no mutagenic ability. As noted above in relation to acute toxicity testing in the same study, mogroside V concentration in the luo han guo extract used is unknown.

Matsushima (1999) completed a series of reverse mutation Ames tests using five strains of *Salmonella typhimurium* and one strain of *Escherichia coli* with a S9 from rat livers. The tests were carried out in conformance with the Japanese Ministry of Health, Labor and Welfare's guidelines for food additive testing procedures. At doses up to and including 5000 µg, luo han guo was confirmed to be nonmutagenic. As the luo han guo extract was a commercial-grade product, the mogroside V concentration would likely have been 30–40%.

3.2.6 Reproductive and developmental toxicity

There have been no studies completed on the reproductive or developmental toxicity of luo han guo extract or mogroside V. Given the extensive history of use of luo han guo (fruit) in China, and luo han guo extract in Japan—as discussed in Section 4.3—any such toxicological effects would likely have been observed in consumer populations already.

Saraya also believes the other toxicity endpoints discussed within this section should be sufficient for FSANZ to complete a satisfactory risk assessment, especially as all of the available toxicity studies resulted in no observed adverse effects even at the highest doses.

3.2.7 Summary

A summary of the NOAEL established for each toxicity category is given in Table 3.1 on the following page.

3.2.8 Human studies

Although there are no human studies available in the published literature, the GRAS determination for BioVittoria's PureLo® luo han guo extract (USFDA 2010) cites two unpublished studies by Xu and Liang. These studies examined the potential pharmacological effects of luo han guo extract in humans, specifically blood sugar response and the effect on liver enzymes. In the first study—assessing blood sugar response—10 subjects were administered luo han guo extract (30–35% mogroside V) in a single dose of 200 mg/kg bw. In the second study—assessing the effect on liver enzymes—six subjects were administered the same single dose of 200 mg/kg bw. Adverse effects were not reported in the summary given in the GRAS determination.

Table 3.1: Summary of toxicity data

Toxicity type	NOAEL for luo han guo extract	NOAEL for mogroside V	References
Acute toxicity	10 g/kg bw	2 g/kg bw	Lee (1975), Makapugay et al. (1985)
Short-term toxicity	7.48 g/kg bw per day	2.92 g/kg bw per day	Marone et al. (2008)
Long-term toxicity	3.2 g/kg bw per day	1.2 g/kg bw per day	Jin et al. (2007), Qin et al. (2006)
Genotoxicity	2 g/kg bw	600 mg/kg bw	Shirasu (1990)
Mutagenicity	Found to be nonmutagenic in microbial systems		Hussain et al. (1990), Matsushima (1999)
Reproductive toxicity	No data	No data	
Developmental toxicity	No data	No data	

3.3 Safety assessment reports prepared by other agencies

As discussed in detail in Section 1.8, luo han guo extract is approved for use in the US, Canada, Japan and China, but is yet to have been assessed by an international body like the JECFA.

In the case of Canada, the regulator, Health Canada, performed an independent assessment of the safety of luo han guo extract under a regulatory environment somewhat similar to that of Australia and New Zealand.

In the US, the four GRAS determinations submitted to the USFDA were all reviewed by an independent panel of food safety experts prior to approval, and approved without further questions for the applicants.

For both Japan and China, a case-by-case safety assessment was not required for luo han guo extract, as it had a demonstrated existing use by the time legislation on such food additives was introduced.

Part 4

Dietary exposure information

4.1 Proposed food groups to contain luo han guo extract

A list of the food groups proposed to contain luo han guo extract is given in Table 4.1 on the next page along with the proposed maximum concentration of luo han guo extract and mogroside V for each proposed food group. The food group names and numbers align with those defined in Schedule 15 – 'Substances that may be used as food additives'.

4.1.1 The percentage of the market likely to use luo han guo extract

Predicting the percentage of market share likely to be gained by luo han guo extract in Australia and New Zealand is a non-trivial task with potential for high relative error in the prediction. Therefore, Saraya suggests to FSANZ a range of methodologies for establishing this parameter in the dietary exposure assessment, given below from least conservative to most conservative.
Table 4.2 on page 37 summarises the market share levels for each method.

Method A—least conservative

Luo han guo extracts share a number of similarities with the steviol glycoside extracts: both are relatively new products in Western markets; both have a long history of use in Japan and (in their unprocessed form) in their native countries; and both are of botanical origin and are typically represented in marketing as 'natural sweeteners' to differentiate them from existing artificial sweeteners. Therefore, it is not inconceivable that luo han guo extract will reach the same market share currently held by steviol glycosides. In the world market for all intense sweeteners across all food group uses, steviol glycosides have a 6% market share (Jolly, 2014).

The figure of 6% could be applied directly to the tabletop sweetener food group for luo han guo extract market share. However, for the other proposed

Table 4.1: Food groups proposed to contain luo han guo extract and the maximum proposed concentrations

Food group name and number	Maximum luo han guo extract concentration, mg/kg	Maximum mogroside V concentration, mg/kg
4.3.4 Fruit and vegetable spreads including jams, chutneys and related products	1100	440
5 Confectionery	1000	400
6.3 Processed cereal and meal products	1000	400
6.4 Flour products	1000	400
7.2 Biscuits, cakes and pastries	1000	400
11.4 Tabletop sweeteners	8000	3200
13.5 Food for special medical purposes	1000	400
20.2.0.3 Dairy and fat based desserts, dips and snacks	1000	400
20.2.0.4 Sauces and toppings	5000	2000

Table 4.2: Market share of luo han guo extract by food group

Food group	Method A	Method B	Method C
Tabletop sweeteners	6%	30%	100%
All other food groups	<1%	3%	100%

food groups—confectionery, biscuits and cakes, sauces and toppings, and jams, chutneys, etc.—the market share that low joule products represent within those groups also needs to be considered. In the FSANZ assessment of neotame for use as an intense sweetener (application A406), this was assumed to be 5–10% for most food groups. Using a market share for low joule products of 10%, and a intense sweetener market share of 6% for luo han guo extract gives an overall market share in these food groups for luo han guo extract-containing products of less than 1%.

Method B—somewhat conservative

As above, considering the similarities between luo han guo extract and steviol glycosides, it might be more appropriate to use the same market share assumed by FSANZ in the assessment of steviol glycosides for use as intense sweeteners (application A540). In that assessment, FSANZ had two scenarios for dietary exposure: a 30% market share based upon a JECFA prediction of future market share; and a complete sugar replacement scenario for the food groups proposed. The 30% market share scenario was acknowledged as more realistic, and used again in the subsequent assessment for increasing the permitted use levels of steviol glycosides (application A1037).

Therefore, it would be equally valid to use the 30% market share scenario for the case of luo han guo extract. Again, this would apply directly to tabletop sweeteners, and assuming a 10% market share for low joule products in other food groups would give a 3% market share for luo han guo extract in those food groups.

Method C—most conservative

Given that an individual consumer may become brand loyal (or, 'ingredient loyal'—preferring one intense sweetener type over others regardless of brand), it is possible that such a consumer would replace all of their consumption of products within the listed food groups with luo han guo-containing products of the same food group. There is also the possibility that this consumer lies in the 90th percentile consumption level for all of those food groups, which would result in the highest possible exposure to luo han guo extract of any individual.

To capture this extreme case, the most conservative assessment methodology would be to assume 100% of all food groups listed contain luo han guo extract at the proposed maximum concentration. While this is not a realistic scenario, it would give a highly conservative estimate of exposure.

4.2 Likely level of consumption

All of the proposed food groups to contain luo han guo extract are included in the most recent Australian and New Zealand National Nutrition Surveys.

Although FSANZ has access to the entire data set, the publicly published data summaries only contain mean and median intake data. This is unfortunately of no use in determining the doses that high-consumption population groups may be exposed to.

So to provide a comparison for the calculations that FSANZ will make in their determination of an ADI using the full data set of the National Nutrition Surveys, here we will consider instead the EDIs used in the four GRAS determinations submitted to the USFDA, as they all provided data for the expected 90th percentile exposure for a variety of population groups, given in Table 1.1 on page 11.

The highest 90th percentile EDI for exposure to mogroside V calculated amongst the GRAS determinations was 3.86 mg/kg bw per day for a healthy child (USFDA 2011). Comparing this to the toxicity data in Section 3.2 gives a 150-fold safety factor over the lowest NOAEL identified for any form of toxicity (600 mg/kg bw for genotoxicity), and a 300-fold safety factor over the long-term toxicity NOAEL for mogroside V.

It is also worth noting that 3.86 mg/kg bw per day for a 75 kg person (conservatively high considering the exposure was for a child) would equate to a daily intake of 290 mg of mogroside V. As the concentration of mogroside V in the raw luo han guo fruit can be up to about 1.4% (Li et al., 2014) and an individual fruit has a mass of around 20 g, a single fruit contains around 280 mg of mogroside V. Therefore, the highest expected daily intake of mogroside V from luo han guo extract consumption in the GRAS determinations would be approximately equivalent to that of a single luo han guo fruit. This level of consumption is consistent with the traditional use of dried luo han guo fruit in China, as discussed in the following section.

4.3 Use of luo han guo extract in other countries

4.3.1 Traditional use of luo han guo

Prior to the work of Lee (1975) in extracting and isolating the sweet compounds of luo han guo and establishing the possibility of using the extract as an intense sweetener, the fruit itself has been used for many centuries in China in the preparation of beverages, especially for use as a traditional medicine. References in Chinese literature of the medicinal uses of luo han guo are reported to date back as far as the 9th century (Fry, 2012). By the 20th century consumption of luo han guo had become widespread, particularly in southern China, and the fruit started becoming of interest to locally-based botanists. A search for the source of the fruit eventually resulted in Swingle's initial botanical description of the plant in 1941.

The traditional use of the fruit was as a remedy for common colds, sore-throats, indigestion and other stomach complaints (Swingle, 1941). The preparation method is typically boiling the dried fruits in water to produce a

Figure 4.1: Dried 'block' form of luo han guo

decoction, drunk as a herbal tea-like beverage (Lee, 1975). The quantity consumed for these purposes is typically one or two dried fruits, twice per day (Dharmananda, 2004).

Prior to industrialisation, around 1000 tons per year of luo han guo (fruit) were consumed for these traditional uses (Swingle, 1941), primarily in southern China.

In the latter half of the 20th century a dried, powdered and compressed 'block' form of luo han guo, as shown in Figure 4.1, became popular and is now commonly found across China and in Asian grocery stores around the world. Such preparations of luo han guo usually have around 5% cane sugar added (Zhu, 1989) and are especially popular in foreign markets for the Chinese diaspora, due to the compact form, convenience, and longer shelf-life. One block represents around one piece of fruit and it is dissolved in boiling water for consumption as a tea similar to the use of the dried whole-fruit.

Although consumption rates of luo han guo in Australia and New Zealand are unknown, the large populations of Chinese migrants and their descendants result in a probably significant consumption for the traditional purposes outlined above. The packaged block form is commonly available in Asian supermarkets and grocery stores throughout Australia and New Zealand.

4.3.2 Luo han guo extract use in Japan

Compared with the fruit, the extract of luo han guo has a more recent history of use following its discovery and study through the 1970s and 1980s. The earliest widespread use of luo han guo extract in food is in Japan. Saraya has been selling food products containing luo han guo extract in Japan since 1995. The best-selling product from the range is Lakanto S, a tabletop sweetener and spoon-for-spoon sugar replacement product containing 0.8% luo han guo extract and 99.2% erythritol. Over the years the range of products has expanded to include jams, marmalade, boiled sweets, curry mixes, cooking sauces and umeshu (plum liqueur). Figure 4.2 shows a selection of Saraya's products with luo han guo extract.

(a) Lakanto S, a tabletop sweetener and sugar replacement

(b) Lakanto blueberry jam

(c) Lakanto White, a tabletop sweetener in ready-to-use sachets

(d) Lakanto sukiyaki sauce

(e) Lakanto mushroom curry mix

(f) Lakanto strawberry flavour boiled sweets

(g) Umeshu (plum liqueur)

Figure 4.2: A selection of Saraya's range of Japanese products with luo han guo extract

For the tabletop sweetener and sugar replacement products, Saraya produces around 700 tonnes per year for the Japanese market alone. This represents around 1.8 million units sold per year in packaged sizes ranging from 75 g to 3 kg.

The remaining products (jams, boiled sweets, etc.) total around 150 tonnes of production per year for the Japanese domestic market, with sales of around 2.1 million units per year.

On all Saraya products there is a phone number to provide feedback or complaints, all of which are recorded in a database. The feedback line receives around 500 calls per year relating to luo han guo extract-containing products. In the 20 years Saraya has been selling these products there has not been a single report of adverse effects attributed to the consumption of luo han guo extract-containing products.

4.3.3 Luo han guo extract use in other countries

Saraya has also been selling luo han guo extract-containing food products in the US since 2007, Canada since 2014 and China since 2015.

In the US and Canada there are a wide variety of products from many manufacturers available. Brands of tabletop sweetener include Saraya's Lakanto (branded as 'Lakanto Monkfruit Sweetener'), Monk Fruit In The Raw, Norbu, and Health Garden Monk Fruit Sweetener. There are many products in other food groups also containing luo han guo extract, including mainstream products by Starbucks, Chobani and the Dole Food Company.

Actual consumption figures are difficult to obtain as most market analyses of intense sweeteners place luo han guo extract in the category of 'other', owing to its relatively low market share in these countries—probably 1% or less. Furthermore, although such market or industry analyses show a history of use of luo han guo extract as an intense sweetener, they do not provide any detail on the number of consumers and so are not useful for establishing individual exposure levels.

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Appendix B

Impurity analysis report

The following two pages contain an English translation of an inspection report issued by the Tokyo Metropolitan Institute of Public Health for an impurity and pesticide residue analysis of a typical batch of luo han guo extract.

Heavy metals		
Cadmium	Not more than 0.1 μg/g	Pass
Chromium	Not more than 2 μg/g	Pass
Lead	Not more than 1 μg/g	Pass
Heavy metal	Not more than 20 μg/g as Pb	Pass
Mercury	Not more than 0.01 μg/g	Pass
Arsenic	Not more than 0.5 μg/g as As	Pass
Chloride	Not more than 1% as NaCl	Pass
Residues after ignition	5.5%	Pass
Ethyl Acetate	Not more than 12.5 μg/g	Pass
Acetone	18 μg/g	Pass
Hexane	Not more than 6.25 μg/g	Pass
Ethanol	Not more than 12.5 μg/g	Pass
Diethylether	Not more than 12.5 μg/g	Pass
Isopropanol	Not more than 12.5 μg/g	Pass
Dichloroethane	Not more than 7.5 μg/g	Pass
Dichloromethane	Not more than 7.5 μg/g	Pass
Trichloroethylene	Not more than 7.5 μg/g	Pass
Methanol	Not more than 12.5 μg/g	Pass
Methyl Acetate	Not more than 12.5 μg/g	Pass
Methylethylketone	Not more than 12.5 μg/g	Pass
Cyclohexane	Not more than 12.5 μg/g	Pass
Bromine	9 μg/g	Pass
Oxamyl	Not more than 0.1 μg/g	Pass
Methomyl	Not more than 0.1 μg/g	Pass
Bendiocarb	Not more than 0.1 μg/g	Pass
Carbaryl	Not more than 0.1 μg/g	Pass
Ethiofencarb	Not more than 1 μg/g	Pass
Isoprocarb	Not more than 0.1 μg/g	Pass
Fenobucarb	Not more than 0.1 μg/g	Pass

Translated by Y. Suzuki for Saraya Co. Ltd.

Methiocarb	Not more than 0.1 μg/g	Pass
Aldicarb sulfoxide	Not more than 0.1 μg/g	Pass
Ethiofencarb sulfoxide	Not more than 0.1 μg/g	Pass
Aldrin	Not more than 0.1 μg/g	Pass
Dieldrin	Not more than 0.1 μg/g	Pass
Endrin	Not more than 0.1 μg/g	Pass
Total BHC	Not more than 0.2 μg/g	Pass
Total DDT	Not more than 0.2μg/g	Pass

Translated by Y. Suzuki for Saraya Co. Ltd.

Appendix D

Application checklists

Two application checklists are included in this appendix: Table D.1 on the following page for general application requirements, and Table D.2 on page 59 for the requirements of an application relating to food additives.

Table D.1: Application checklist—general requirements

	Item	Page	Check	
A	Form of application	-		
	Application in English	-	☑	
	Executive summary (separated from main application)	-	☑	
	Relevant sections clearly identified	-	☑	☑
	Pages sequentially numbered	-	☑	
	Electronic copy (searchable)	-	☑	
	All references provided	60	☑	
B	Applicant details	6		☑
C	Purpose of the application	7		☑
D	Justification for the application	7		
	Regulatory impact information	7	☑	☑
	Impact on international trade	8	☑	
E	Information to support the application	9		☑
	Data requirements	9	☑	
F	Assessment procedure—Major	10		☑
G	Confidential commercial information	10		
	Confidential material separated in application	n/a	⊟	☑
	Formal request including reasons	n/a	⊟	
	Non-confidential summary provided	n/a	⊟	
H	Other confidential information	10		
	Confidential material separated in application	n/a	⊟	☑
	Formal request including reasons	n/a	⊟	
I	Exclusive Capturable Commercial Benefit	10		☑
	Justification provided	10	☑	
J	International and other national standards	10		
	International standards	10	☑	☑
	Other national standards	11	☑	
K	Statutory Declarations	54		☑
L	Checklists provided with application	57		
	Application checklist for general requirements	58	☑	☑
	Application checklist for food additives submission	59	☑	

Table D.2: Application checklist—food additives

	Item	Page	Check
A.1	Nature and technological function information	14	☑
A.2	Identification information	15	☑
A.3	Chemical and physical properties	17	☑
A.4	Impurity profile	17	☑
A.5	Manufacturing process	22	☑
A.6	Specifications	25	☑
A.7	Food labelling	25	☑
A.8	Analytical detection method	25	☑
A.9	Additional functions	26	☑
B.1	Toxicokinetics and metabolism information	28	☑
B.2	Toxicity information	29	☑
B.3	Safety assessments from international agencies	34	☑
C.1	List of foods likely to contain the food additive	35	☑
C.2	Proposed levels in foods	35	☑
C.3	Likely level of consumption	38	☑
C.4	Percentage of food group to contain the food additive	35	☑
C.5	Use in other countries (if applicable)	38	☑
C.6	Where consumption has changed, information on likely consumption	n/a	⊟

Appendix E

Copies of cited references

Copies of all cited references accompany this application in electronic form.

All references are provided in PDF format to ensure the widest compatibility. Where the original source was a website, some formatting may have been lost in the conversion to PDF, however all text is preserved in the converted files.

Given below is a complete list of accompanying references, including notes regarding the PDF file where necessary. For the full citations, see the reference list.

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