APPLICATION

January 2025

TO:

Food Standards Australia New Zealand

IN RELATION TO:

Application to amend Schedule 15 – Substances that may be used as food additives of the Australia New Zealand Food Standards Code

Extension of use to permit up to 350mg/kg steviol equivalents in flour products (including noodles and pasta) [Schedule 15 - 6.4].

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(As per section 3.1.1 B of the Application Handbook as at 1 July 2024)

Applicant Details

(As per section 3.1.1 B of the Application Handbook as at 1 July 2024)

Applicant	[redacted]
Organisation	George Weston Foods Limited (GWF)
Address: office & postal	[redacted]
Telephone	[redacted]
Email address	[redacted]
Primary contact	[redacted]

Nature of Business

(As per section 3.1.1 B of the Application Handbook as at 1 July 2024)

GWF is one of Australia and New Zealand's largest food manufacturers employing more than 6,000 people across 43 manufacturing sites, including a significant presence in regional areas. Additionally, we have a broad supply chain of businesses that we partner with to deliver our products.

Our products are diverse and include leading brands such as Tip Top, Abbott's Bakery, Burgen, Golden, Big Ben, Dad's Pies, DON Smallgoods and Yumi's. Through our MAURI business, we are one of Australia's leading suppliers of flour, bakery ingredients and animal foods. We support parts of the local operations for other brands on behalf of our parent company.

GWF is a wholly owned subsidiary of Associated British Foods Plc (ABF), a diversified international food, ingredients, and retail group with operations in 52 countries across Europe, Southern Africa, the Americas, and Asia Pacific.

GWF has been part of the food landscape in Australia and New Zealand since the 1930's.

Details of other parties associated with the Application

(As per section 3.1.1 B of the Application Handbook as at 1 July 2024)

The following Scientific and Regulatory Consultant is involved in the preparation, submission, and stewardship of this application:

[redacted]

1. Application Information

Terminology

For the purpose of this application document, the different forms of steviol glycosides will be referred to collectively as steviol glycosides (SG).

Status of Similar Applications

(As per Section 3.1.1 D of the Application Handbook as at 1 July 2024)

GWF is not aware of any similar current applications.

Assessment Procedure

(As per section 3.1.1 F of the Application Handbook as at 1 July 2024)

GWF seeks to proceed with an **unpaid** application for consideration as a General Procedure, Level 2 (maximum total variable hours: 290 hours). SG have already been assessed by FSANZ and are already permitted in several food categories.

Confidential commercial information [CCI]

(As per section 3.1.1 G of the Application Handbook as at 1 July 2024)

GWF is providing information to support this application which it considers to be confidential commercial information (CCI).

This information is provided separately and is clearly labelled as CCI.

Other confidential information

(As per section 3.1.1 H of the Application Handbook as at 1 July 2024)

This application does not include any other confidential information, aside from the confidential commercial information noted above.

Exclusive capturable commercial benefit [ECCB]

(As per section 3.1.1 I of the Application Handbook as at 1 July 2024)

This application will **not** confer an exclusive capturable commercial benefit (ECCB) for GWF or any other individual company.

The permission to voluntarily add SG to flour products (category 6.4 in Schedule) would be available to all manufacturers.

Although the manufacturers of steviol glycosides will derive some economic benefit from approval of the application, other food manufacturers who use steviol glycosides may also derive some economic benefit.

In addition, there are several different manufacturers of steviol glycosides and therefore the economic benefit could be spread and not be exclusive.

Statutory declaration

(As per section 3.1.1 K of the Application Handbook as at 1 July 2024)

A statutory declaration has been provided with this application.

Checklist

(As per section 3.1.1 L of the Application Handbook as at 1 July 2024)

Completed checklists for sections 3.1.1 (General requirements), 3.2.1 (General Information to support the proposed labelling change) and 3.3.1 (Food Additives) of the Application Handbook are provided with this application.

2. Purpose of the Application

(As per section 3.1.1 C of the Application Handbook as at 1 July 2024)

GWF is making this application to request an amendment to the *Australia New Zealand Food Standards Code* (hereafter, the Code) to extend the permitted use of steviol glycosides to flour products that are cooked on hot plates - crumpets, pikelets, and pancakes (Schedule 15 - category 6.4) up to a maximum level of 350mg/kg steviol equivalents.

SG are already permitted for use in several food product categories in accordance with Standard 1.3.1 and Schedule 15 - this application is requesting an extension of the current permissions to an additional food category.

The function of steviol glycosides (INS 960) as an intense sweetener in hotplate products will be to provide technical functionality by enabling the manufacture of these products with improved/increased sweetness without the use of (additional) added sugar which results in sticking, causing waste on the production line.

The Applicant has determined that SG are the preferred alternative to other available and permitted artificial sweeteners.

The following is provided as an example of an amendment that would achieve the purpose of this Application.

S15-5 Table of permissions for food additives			
6.4 - Flour products (including noodles and pasta)			
INS (if any)	*Description*	*MPL*	*Conditions*
960	Steviol glycosides	350mg/kg	-

3. Justification for the Application

(As per section 3.1.1 D of the Application Handbook as at 1 July 2024)

3.1 Need for the Proposed Change

(As per section 3.1.1 D(a) of the Application Handbook as at 1 July 2024)

SG meet the Standard 1.1.2 definition of a substance that is used as a food additive as it is:

- identified in Schedule 15 as a substance that may be used as a food additive; and
- is added to food to perform a technological purpose listed in Schedule 14 as a sweetener.

Flour products (including noodles and pasta) are permitted to contain food additives listed at item 6.4 of the table to Section 15-5 of Schedule 15 of the Code. Item 6.4 does not currently list SG as a permitted food additive.

The amendment requested is therefore required to establish permission to add SG to hotplate products which sit under category 6.4 - Flour products (including noodles and pasta) of Schedule 15 to:

1. provide a feasible technical solution to enable the applicant to manufacture crumpets with a sweeter taste profile than is currently available in the GWF product portfolio without compromising on processing capabilities and nutrition profile.
2. provide the applicant with the means to reduce sugar content in pikelets and pancakes improving the overall nutrition profile of these products.

Hotplate Products

The term **hotplate product** refers to a type of food prepared by cooking batter directly on a flat, heated surface such as a hotplate, griddle, or skillet. These products typically have a distinct texture and appearance due to their cooking method, which involves heat applied from below without the use of an oven.

They include:

- Crumpets: round, spongy products with a porous top surface, made from a yeast or chemically leavened batter. The holes form naturally as the batter cooks, creating a texture ideal for absorbing toppings.
- Pancakes: flat, round, and often slightly fluffy products made from a non-yeast batter, typically cooked on both sides.
- Pikelets: like pancakes but smaller and thinner, common in the UK and Australia.

GWF have been manufacturing Golden Crumpets since 1959 followed by Golden Pikelets and Pancakes in the 80s. GWF are currently the only large-scale manufacturer of hotplate products within ANZ and have built up significant technical expertise in the development and manufacture of these products. Golden crumpets, pikelets and pancakes are manufactured in every state in Australia and in New Zealand.

Crumpets

Crumpets are most associated with the United Kingdom (UK), where they originated, however they are enjoyed in several other countries, particularly those influenced by British culinary traditions. While crumpets are most prominent in the UK, Australia, and New Zealand, their availability in other regions often depends on British expatriate communities or cultural influence.

The applicant wishes to expand the existing crumpet portfolio with the addition of sweet crumpets without compromising on processing capabilities and nutrition profile.

Crumpets are a unique product by formulation and process. They are a high moisture flour-based product with a non-acid pH (6-8) and high water activity (0.95-0.97). Crumpets are savoury by nature due to their elevated sodium content due to the chemical leavening system used in the manufacture of these products.

The GWF manufacturing process for crumpets has not changed in 60 years - they are cooked in cast iron pots over naked gas flames at around 180^0C for 3 minutes then passed under a grill to set the top of the crumpet. The cast iron pots are not oiled or lubricated in any way and use heat to release product from the pots. This is an extremely quick cook process compared to other bakery products.

The applicant's current crumpet range of plain and wholemeal varieties are savoury products with a high sodium content due to the chemical leavening system used in the manufacture of these products. Sodium content is shown in Table 1 below. A combination of pyrophosphates (450) and sodium carbonates (500) is used for aeration, giving the traditional 'holes' associated with these products. There is no added sucrose.

Table 1: Sodium content of Golden crumpets[1]

Variety	Sodium per serve (1 crumpet)	Sodium per 100g
Golden Plain - round	335mg	670mg
Golden Wholemeal - round	328mg	655mg

During the manufacture of crumpets, no processing oil is used on production lines as a release agent. Addition of sugar results in the crumpets sticking to the hotplate equipment causing damage to the product, waste and production delays and down time. These technical challenges are limiting the opportunity to develop sweet crumpet product line extensions.

Through trials the applicant has determined the sugar addition rate threshold sits at 1% of total formulation - anything higher causes the batter to stick in the hotplate pots. With sugar addition at this level, product does not release on completion of cooking creating waste and production downtime.

The applicant has investigated alternative ways to improve the sweetness profile of the formulations outside of sugar addition at the level required. Through product development trials steviol glycosides (SG) have been found to provide all the attributes required to produce sweet crumpets.

[1] Source: Tip Top website, accessed 30/12/2024

Historically the applicant has trialed other sugar substitutes such as sugar alcohols and natural sweeteners such monk fruit with no success due to either not meeting sensory and nutritional profiles or processing issues due to product sticking on the production line.

SG deliver on both organoleptic characteristics and processing requirements as well as maintaining or enhancing product nutritional profiles.

Pikelets and pancakes

Hotplate products such as pikelets and pancakes have added sugar and canola oil as part of the formulation. They are processed on an open hotplate that is oiled for release.

The ability to add SG to these products will provide the applicant with the means to reduce sugar content where necessary, improving the overall nutrition profile of these products.

Overall

Permission to add SG into crumpet and other hotplate products presents an opportunity to expand the applicant's portfolio to include products with improved nutrition profiles and manage production waste streams.

Alternative sweeteners do not provide the technical outcomes required.

SG in crumpets and hotplate products demonstrate the highest level of processing stability in addition to being stable when exposed to light[2], and heat and pH stable in preparation when compared to other sweetener alternatives trialed.

The requested addition rate of up to 350mg/kg steviol equivalents has been determined through a rigorous product development process to achieve the specified purpose and has been proven to be stable through the short shelf life of these products.

[2] an advantage of SG during production as ingredients are hand weigh and the SG will be exposed to light.

3.2 Advantages of the Proposed Change

(As per section 3.1.1 D(b) of the Application Handbook as at 1 July 2024)

Currently the Code permits multiple artificial sweeteners such as acesulphame potassium (950), alitame (956) and aspartame-acesulphame salt (962) to be added to flour products.

Additionally, sugar alcohols including erythritol (968), sorbitol (420), mannitol (421) are permitted to be added to flour products as GMP additives.

In the climate of consumers being more health conscious, the applicant believes that SG are a preferable alternative to these artificial sweeteners.

The change proposed will allow manufacturers of hotplate products to develop and market products with improved nutrition profiles.

For example, Tables 2 - 4 below show the nutrition information for crumpets, pikelets, and pancakes with and without SG.

With respect to crumpets, the use of SG improved the Health Star Rating (HSR) from 3.0 to 3.5.

With respect to pikelets and pancakes, the use of SG reduced the sugars per 100g by over 50%.

Table 2: Nutrition information - crumpets with and without SG

Nutrient	Crumpets with SG [1]		Standard Crumpets	
	Average Quantity per 50g serving (1 crumpet)	Average Quantity per 100g	Average Quantity per 50g serving (1 crumpet)	Average Quantity per 100g
Energy	347 kJ	695 kJ	398 kJ	795 kJ
Protein	2.6 g	5.2 g	3.2 g	6.3 g
Fat, total	0.3 g	0.6 g	0.7 g	1.3 g
- saturated	0.1 g	0.1 g	0.1 g	0.2 g
Carbohydrate	16.7 g	33.4 g	18.5 g	37.0 g
- sugars	LESS THAN 1 g	LESS THAN 1 g	1.0 g	2.0 g
Sodium	242 mg	484 mg	335 mg	670 mg
HSR [2]		3.5		3.0

[1] Nutrition information calculated using the FSANZ Nutrition Panel calculator. [2] HSR calculated using the HSR calculator 4.2.

Table 3: Nutrition information - pikelets with and without SG

Nutrient	Pikelets with SG [1]		Standard Pikelets	
	Average Quantity per 25g serving (1 pikelet)	Average Quantity per 100g	Average Quantity per 25g serving (1 pikelet)	Average Quantity per 100g
Energy	240 kJ	961 kJ	248 kJ	990 kJ
Protein	1.4 g	5.8 g	1.4 g	5.5 g
Fat, total	1.3 g	5.1 g	1.5 g	6.0 g
- saturated	0.2 g	0.7 g	0.2 g	0.6 g
Carbohydrate	9.7 g	38.8 g	9.6 g	38.2 g
- sugars	1.4 g	5.4 g	3.0 g	11.9 g
Sodium	75 mg	300 mg	94 mg	375 mg
HSR		3.5		3.5

[1] Nutrition information calculated using the FSANZ Nutrition Panel calculator. [2] HSR calculated using the HSR calculator 4.2.

Table 4: Nutrition information - pancakes with and without SG

Nutrient	Pancakes with SG [1]		Standard Pancakes	
	Average Quantity per 60g serving (1 pancake)	Average Quantity per 100g	Average Quantity per 60g serving (1 pancake)	Average Quantity per 100g
Energy	576 kJ	961 kJ	578 kJ	963 kJ
Protein	3.5 g	5.8 g	3.4 g	5.6 g
Fat, total	3.1 g	5.1 g	3.6 g	6.0 g
- saturated	0.4 g	0.7 g	0.4 g	0.6 g
Carbohydrate	23.3 g	38.8 g	23.1 g	38.5 g
- sugars	3.3 g	5.4 g	7.3 g	12.2 g
Sodium	180 mg	300 mg	180 mg	300 mg
HSR		3.5		3.5

[1] Nutrition information calculated using the FSANZ Nutrition Panel calculator. [2] HSR calculated using the HSR calculator 4.2.

3.3 Disadvantages of the Proposed Change

(As per section 3.1.1 D(b) of the Application Handbook as at 1 July 2024)

The Applicant has not identified any disadvantages of the proposed change.

3.4 Public Health and safety issues

(As per section 3.1.1 D of the Application Handbook as at 1 July 2024)

The Applicant has not identified any public health and safety issues in relation to the proposed change.

The Code currently permits the use of SG as food additives with the INS number 960. They are permitted in a wide range of food categories listed within the table to section S15—5 at specified maximum permitted levels (MPL) and at Good Manufacturing Practice (GMP) for tabletop sweeteners only.

A list of current permissions in the Code for SG is set out in Table 6.

Refer **Sections 7.2 and 7.3** for information related to the safety of SG.

3.5 Consumer choice

(As per section 3.1.1 D of the Application Handbook as at 1 July 2024)

The Applicant has not identified any consumer choice issues in relation to the proposed change.

Under existing labelling requirements in the Code (unless the food is exempt from the requirement for a statement of ingredients) SG require declaration as a food additive in the statement of ingredients using the food additive name 'steviol glycosides' or the International Numbering System (INS) code number 960 (as listed in Schedule 8).

Consumers will be able to make an informed choice to purchase and include hotplate products containing SG in their diets based on the declaration of SG in the ingredient list.

3.6 Support for the proposed change

(As per section 3.1.1 D of the Application Handbook as at 1 July 2024)

The Applicant is not aware of any direct evidence that other manufacturers have an interest in, or support, the proposed change. However, given the need for the amendment requested, the advantages described above demonstrate there is a market opportunity, and the applicant anticipates other manufacturers will be supportive of the proposed changes which facilitate innovation in the category.

The requested amendment is voluntary so manufacturers will be able to choose whether they take up the opportunity to produce hotplate products containing SG for their consumers.

4 Regulatory Impact Information

(As per section 3.1.1 D.1 of the Application Handbook as at 1 July 2024)

4.1 Costs and Benefits of the Application

(As per section 3.1.1 D.1.1 of the Application Handbook as at 1 July 2024)

4.1.1 Costs and Benefits – Consumer

The requested amendment MAY result in an increased purchase price for these products over existing products. The applicant considers the pricing for these products would not exceed the maximum price range in market of that category at the time of launch.

However, as noted, these products will be a range extension and consumers can therefore choose to purchase these new products or continue to purchase existing products.

Consumers will be able to make an informed choice to purchase products containing SG based on the declaration of the additive in the ingredient list, nutrition information and HSR.

The advantages for consumers were outlined in Section 3.2.

4.1.2 Costs and Benefits - Industry and Business

The requested amendment is a voluntary permission to add SG to flour products. Manufacturers can make a choice as to whether they will take up the opportunity to offer consumers hotplate products containing SG.

The costs for manufacturers of hotplate products who choose to develop and market them include investment in ingredient costs and the manufacturing process to achieve inclusion of SG into the product.

The benefit to industry is the ability to offer a wider choice of products to consumers. This allows industry to continue to innovate and provide alternative offerings to consumers.

Industry will benefit from having an additional intense sweetener (food additive) available to them. Due to the voluntary nature of the permission, industry will only use the food additive where they believe a net benefit exists.

4.1.3 Costs and Benefits – Government

As SG are already approved for food use within Australia and New Zealand, there is no regulatory cost to Government beyond:

- the initial regulatory cost of approval
- making compliance officers aware of the new permission, and
- the normal costs of enforcing compliance with the Code.

4.2 Impact on International Trade

(As per section 3.1.1 D.1.2 of the Application Handbook as at 1 July 2024)

The Applicant notes that, in developing food standards, FSANZ must have regard to its WTO obligations; the promotion of consistency between domestic and international food standards; and the promotion of fair trading in food. These matters encompass consideration of international standards and trade issues.

Approval may also provide additional markets for SG manufactured in markets outside of Australia and New Zealand by increasing the range of product categories permitted to contain SG in the ANZ market.

5 Information to Support the Application

(As per section 3.1.1 E of the Application Handbook as at 1 July 2024)

5.1 Data Requirements

(As per section 3.1.1 E.1 of the Application Handbook as at 1 July 2024)

The data and information provided to support the application fulfils the requirements for data as set out in the FSANZ Application Handbook:

- the source, author and year of evidence is identified
- it has been obtained using validated methods.
- it represents Australian and New Zealand populations where possible, and
- it has been compiled from studies conducted under good laboratory practice (GLP).

FSANZ has assessed information relating to the use of SG as a food additive on numerous occasions in the context of previous applications to amend the Code. This application refers to supporting information that has already been assessed by FSANZ and additional information that has been published in 2024.

Refer to **Section 7** for information about the food additive – steviol glycosides.

5.2 FSANZ Act Objectives

Information is provided in this Application to address the objectives specified in Section 18 of the FSANZ Act 1991 as follows:

Protection of public health and safety (1a)
This is addressed in Sections 7.2 and 7.3 of this application document.

The provision of adequate information relating to food to enable consumers to make informed choices (1b)
The existing labelling requirements in the Code will apply to flour products with added SG to assist consumers to make informed choices.

The prevention of misleading or deceptive conduct (1c)
The existing labelling requirements applying to foods containing added SG will apply to flour products.

The need for standards to be based on risk analysis using the best available scientific evidence (2a)
This is addressed in Sections 7.2 and 7.3 of this application document.

The promotion of consistency between domestic and international food standards (2b)
SG added to foods for retail sale in Australia and New Zealand must meet specifications as set out in Schedule 3 of the Code.

The desirability of an efficient and internationally competitive food industry (2c)
Permission to add plant SG to flour products would facilitate alignment with international permissions.

The recommended approach will also allow for more innovation and increased market opportunities generally.

5.3 Policy Guidelines

(As per section 3.3.2 of the Application Handbook as at 1 July 2024)

Addition to Food of Substances other than Vitamins and Minerals

The Policy Guideline 'Addition of Substances other than Vitamins and Minerals' includes specific order policy principles for substances added to achieve a solely technological function, such as food additives.

These specific order policy principles state that permission should be granted as per the principles set out in Table 5.

Table 5: Specific Order Policy Principles

Specific Order Policy Principles – Any Other Purpose	Section of Application
a) the purpose for adding the substance can be articulated clearly by the manufacturer (i.e., the 'stated purpose'); and	Sections 2 and 7.1
b) the addition of the substance to food is safe for human consumption; and	Sections 7.2 and 7.3. The addition of SG to flour products at the levels proposed is safe.
c) the substance is added in a quantity and a form which is consistent with delivering the stated purpose; and	Section 7.1. The form of the SG is consistent with the stated purpose and already permitted in the Code.
d) the addition of the substance is not likely to create a significant negative public health impact to the general population or sub population; and	Section 7.2 and 7.3 There are no adverse health effects associated with the addition of SG to flour products as proposed.
e) the presence of the substance does not mislead the consumer as to the nutritional quality of the food.	Products containing added SG are already labelled in a manner which informs and does not mislead consumers regarding the nutritional quality of the food. SG will be declared in the ingredient list and the NIP will reflect the nutrition status of the product. No change to current labelling requirements is proposed.

6 International and Other National Standards

(As per section 3.1.1 J of the Application Handbook as at 1 July 2024)

6.1 International Standards

(As per section 3.1.1 J.1 of the Application Handbook as at 1 July 2024)

6.1.1 Codex Alimentarius General Standard for Food Additives (GSFA)

The Codex Committee on Food Additives (CCFA) adopted permissions for the food additive 'steviol glycosides' (with the food additive number of INS 960) as a sweetener in 2011 for a wide variety of food categories in the Codex Alimentarius General Standard for Food Additives (GSFA).

The GSFA provisions for SG are provided in **Appendix 1.** Approvals include like products such as:

- Bread and ordinary bakery wares (07.1) – max level – 165mg/kg
- Fine bakery wares (sweet, salty, savory) and mixes (07.2) – max level – 350mg/kg[3]

6.1.2 JECFA (Joint FAO/WHO Expert Committee on Food Additives)

The most recent Specifications Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 96th Meeting - (Framework for) Steviol glycosides was published in FAO Monographs 31 (2023) and contains four (4) annexes covering the four methods of production as well as method of assay details. This is provided as **Appendix 2.**

An ADI of 0 – 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008). This has not changed with the release of the 2023 Specifications Monographs.

[3] https://www.fao.org/gsfaonline/groups/details.html?id=309&print=true, accessed 30/12/2024.

6.2 Other National Standards or Regulations

(As per section 3.1.1 J.2 of the Application Handbook as at 1 July 2024)

6.2.1 Australia/New Zealand

Table 6 sets out the current permissions and MPLs in the Code for SG in food products and the requested level for flour products.

Table 6: Current Steviol glycoside permissions in the Code

Food	S15-5 Class Code	MPL mg/kg	Conditions for SG addition
Liquid milk products and flavoured liquid milk	1.1.2	115	
Fermented milk products and rennetted milk products	1.2.2	175	
Ice cream and edible ices	3	200	
Fruits and vegetables in vinegar, oil, brine, or alcohol	4.3.2	160	
Low joule chutneys, low joule jams and low joule spreads	4.3.4.1	450	
Fruit and vegetable preparations including pulp	4.3.6	210	
Chocolate and cocoa products	5.1	550	
Sugar confectionery	5.2	1,100	
Processed cereal and meal products	6.3	250	
Fancy breads	7.1.1	160	
Biscuits, cakes, and pastries	7.2	160	
Tabletop sweeteners	11.4	GMP	
Formulated meal replacements and formulated supplementary foods	13.3	175	
Formulated supplementary sports foods	13.4	175	
Food for special medical purposes – all other	13.5	75	Not for a very low energy food. Not for a product formulated for infants.
Food for special medical purposes – very low energy food only	13.5	330	
Fruit and vegetable juices	14.1.2.1	50	
Fruit drink	14.1.2.2.1	200	
Low joule fruit and vegetable juice products	14.1.2.2.2	125	
Soy bean beverage (plain or flavoured)	14.1.2.2.3	100	Only plain soy bean beverage
		200	Only flavoured soy bean beverage
Water based flavoured drinks	14.1.3	200	
Formulated Beverages	14.1.4	200	
Coffee, coffee substitutes, tea, herbal infusions, and similar products	14.1.5	100	

Food	S15-5 Class Code	MPL mg/kg	Conditions for SG addition
Custard mix, custard powder and blancmange powder	20.2.0.1	80	
Jelly	20.2.0.2	260	
Dairy and fat-based desserts, dips, and snacks (only dairy and fat-based dessert products)	20.2.0.3	150	Only dairy and fat-based dessert products
Sauces and toppings (including mayonnaises and salad dressings)	20.2.0.4	320	
PROPOSED:			
Flour products (including noodles and pasta)	6.4	350	

6.2.2 Other Jurisdictions

This section provides an overview of permissions for SG in other jurisdictions.

Table 7: Summary of Global Regulatory Approvals

Food category(s)	Prescribed form & Permitted level	Comment in relation to this application
European Union		
Currently, steviol glycosides (E 960a–d) are authorised as a food additive in the EU in 32 different food categories (FCs) (representing 42 uses). Additionally, they are permitted for use at QS in table-top sweeteners. The permissions are set out in Annex II to Regulation (EC) No 1333/2008 (as amended by Regulation 1131/2011) Commission Regulation (EU) No. 1131/2011	Steviol glycosides (E 960a–d) MPLs ranging from 20 to 3,300 mg steviol equivalents/kg or L.	The food categories permitted to contain SG contain a category of 'fine bakery wares' with a maximum level of 330mg/kg. There are no other food categories which could cover hotplate products. It should be noted that crumpets are not marketed in the EU to the applicant's knowledge.
United States of America (USA) - GRAS The applicant acknowledges that GRAS is not a regulation however it has been included in this section of the application for completeness. SG are permitted where the vendor provides a GRAS notice covering the intended use or the use of the ingredient aligns with an existing GRAS notice and intended use that has been approved by the US Food and Drug Administration. A search of the US FDA GRAS Notices database identified 63 records dating from 2008 (GRN 000252) – current (GRN 1178, Pending).		
Canada		
Marketing Authorization for Food Additives That May Be Used as Sweeteners 9. List of Permitted Sweeteners (Lists of Permitted Food Additives) S.1.2	Steviol glycosides from: *Stevia rebaudiana* Bertoni; *Saccharomyces cerevisiae* CD15380; *Saccharomyces cerevisiae* CD15407; *Saccharomyces cerevisiae* Y63348; *Yarrowia lipolytica* VRM Maximum level of use ranges from 0.012% - 0.035% calculated as steviol equivalents.	The food categories permitted to contain SG do not include a category that would cover hotplate products. It should be noted that crumpets are not marketed in Canada to the applicant's knowledge.

Food category(s)	Prescribed form & Permitted level	Comment in relation to this application
	There is permission for 'Unstandardized bakery products' to a level of 350ppm in the food as consumed. Note for table top sweeteners the level is GMP.	
Asia		
Singapore		
Singapore [Regulation 18(3) and (4)], Thirteenth Schedule Permitted Sweetening Agents in Selected Foods and Their Maximum Permitted Levels Fruit drinks & Vegetable juice drinks: (a) Product label to carry an advisory statement that children 9 years old and below should not consume more than 2 servings a day, based on serving size of 250 ml.	Steviol Glycosides (as steviol) are permitted at a range of MPLs, including: **Selected Foods:** • Cereals and cereal products – 150 – 330ppm • Snacks – 170ppm	The food categories permitted to contain SG do not include a category that would cover hotplate products. It should be noted that crumpets are not marketed in Singapore to the applicant's knowledge.
Japan		
Food Additives are regulated under the Food Sanitation Act	Stevia extract is listed on the 'List of Existing Food Additives' Permitted in all food categories with no usage limit	
India		
Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011 Stevioside (960) is listed as a non-caloric sweetener. Standards are listed in Chapter 3 of the Food Safety and Standards (Food Products Standards and Food Additives) Regulation 2011.	**Food categories:** Steviol glycosides are permitted in a range of food categories at a range of Recommended max levels. These are set out in Appendix A – Tables 1-15 of the Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011. Including: • Cereals/pulses and starch-based desserts (6.5) – 165mg/kg	The food categories permitted to contain SG do not include a category that would cover hotplate products. It should be noted that crumpets are not marketed in India to the applicant's knowledge.

European Union
Food additives approved in the 27 EU Member States are listed in Annex II of Regulation (EC) No 1333/2008.[4] [5] [6] This Regulation is supported by a database for ease of searching.

In the European Union (EU), commercially available steviol glycoside products must comply with the specifications for steviol glycosides (International Numbering System for Food Additives [INS] No. 960) adopted by the European Commission in 2012 and updated in 2021 and 2022 to include rebaudioside M, Rebaudioside D, and Rebaudioside AM produced through enzymatic conversion[7] and glucosylated steviol glycosides. As such, steviol glycoside products are approved for use in the EU and must contain no less than 95% total steviol glycosides from 11 total named steviol glycosides (dulcoside, rebaudiosides A, B, C, D, E, F, and M, rubusoside, steviolbioside, and stevioside). Within their safety assessment of 2010 (EFSA, 2010), the EFSA Panel corroborated the JECFA conclusion arising from their 69th meeting and an ADI of 0 to 4 mg/kg body weight, as steviol equivalents, was established for steviol glycosides. In 2020, EFSA evaluated the safety of a proposed amendment to the steviol glycoside specification to expand the list of permissible steviol glycosides to include all of those that are present in the leaves of the *S. rebaudiana* Bertoni plant (EFSA, 2020) and thus align the EU specification with that of the current JECFA specification. The EFSA Panel on Food Additives and Flavourings (FAF Panel) noted that all steviol glycosides have been observed to share the same metabolic fate, and ultimately concluded that the 60 steviol glycosides identified within the proposed amendment could be established as safe due to the vast toxicological database that has previously been evaluated for these compounds, as a read-across approach could be undertaken. The existing ADI of 4 mg/kg body weight (as steviol equivalents) would be applicable to all 60 of the identified.

In November 2024, EFSA published a *Scientific opinion on the extension of the authorisation of use of the food additive steviol glycosides (E 960a–d) and the modification of the acceptable daily intake (ADI) for steviol*.

The EFSA Panel on Food Additive and Flavourings (FAF Panel) evaluated the safety of proposed changes to the currently permitted uses of the food additive steviol glycosides (E 960a–d) and of a proposed modification of the current acceptable daily intake (ADI) from 4 mg/kg body weight (bw) per day to 6 or 16 mg/kg bw per day, expressed as steviol equivalents. Currently, steviol glycosides (E 960a–d) are authorised in the EU in 32 different food categories (FCs). An extension of use was proposed for four new uses within FC 7.2 'Fine bakery wares'. In addition, an increase of the maximum permitted levels (MPLs) for FC 14.1.3 'Fruit nectars' and for three uses within FC 14.1.4 'Flavoured drinks' was requested. Consequently, the Panel updated the exposure estimates using

[4] https://food.ec.europa.eu/food-safety/food-improvement-agents/additives/database_en

[5] https://food.ec.europa.eu/food-safety/food-improvement-agents/additives/eu-rules_en

[6] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02008R1333-20231005

[7] Commission Regulation (EU) 2021/1156 of 13 July 2021 amending the Annex to Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards specifications for steviol glycosides (E 960) and rebaudioside M. C/2021/5062. Off J Eur Union 64(L249):87-98. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32021R1156&from=EN.

the protocol for assessing exposure to sweeteners, developed to consider the specificities related to consumers' exposure to this functional class of food additives. Considering the proposed extension of use and increase of the MPLs, together with the currently authorised uses (at the MPLs) of E 960a–d, the highest 95th percentiles of exposure are 4.1 and 6.9 mg/kg bw per day for infants and toddlers, respectively. Based on the currently available absorption, distribution, metabolism, and excretion (ADME) dataset for steviol glycosides (E 960a–d), the Panel concluded that that there is insufficient justification to increase the current ADI of 4 mg/kg bw per day, expressed as steviol equivalents. With respect to the proposed extension of use and increase of the MPLs, the Panel concluded that the calculated, conservative, dietary exposure would result in an increased exceedance of the ADI for toddlers at the 95th percentile (EFSA, 2024)

United States
The applicant acknowledges that GRAS is not a regulation however, consideration of the GRAS history of SG has been included in this section of the application for completeness.

Several GRAS Notices for major individual steviol glycosides, including stevioside, rebaudiosides A, C, D, and X/M, mixtures of steviol glycosides, and glycosylated and enzyme-modified steviol glycosides have been reviewed by the U.S. Food and Drug Administration. A search of the US FDA GRAS Notices database identified 63 records dating from 2008 (GRN 000252) – current (GRN 1178, Pending).[8]

The FDA has consistently raised "no questions" regarding the GRAS status of these steviol glycoside products for use as general-purpose sweeteners in various food and beverage products, suggesting that these products have a general recognition of safety.

The requests are for a range of food products including 'baked goods and baking mixes' which is a general food category under food additives (21 CFR Part 170).[9]

Baked goods and baking mixes, including all ready-to-eat and ready-to-bake products, flours, and mixes requiring preparation before serving.

Hotplate products are not specifically listed in this category.

The intended use levels vary by food category, but the actual levels are self-limiting due to organoleptic factors and consumer taste considerations. Generally, use levels are at 'good manufacturing practice' which is defined as follows:

> *(b) For the purposes of this section, good manufacturing practice shall be defined to include the following restrictions:*
>
> > *(1) The quantity of a substance added to food does not exceed the amount reasonably required to accomplish its intended physical, nutritional, or other technical effect in food; and*
> >
> > *(2) The quantity of a substance that becomes a component of food as a result of its use in the manufacturing, processing, or packaging of food, and which is not intended to accomplish*

[8] GRAS (generally recognized as safe) notifications - Stevia., carried out 24/11/2024.
[9] https://www.ecfr.gov/current/title-21/part-170

any physical or other technical effect in the food itself, shall be reduced to the extent reasonably possible.

(3) The substance is of appropriate food grade and is prepared and handled as a food ingredient. Upon request the Commissioner will offer an opinion, based on specifications, and intended use, as to whether or not a particular grade or lot of the substance is of suitable purity for use in food and would generally be regarded as safe for the purpose intended, by experts qualified to evaluate its safety.[10]

The United States Pharmacopeia Convention Food Chemicals Codex (FCC) contains a specification for steviol glycosides.

Canada
Health Canada published its final clearance for use of steviol glycosides as a sweetener in foods in July 2012. The use of steviol glycosides as a sweetening agent in a variety of food and beverage categories was approved at levels of up to 0.35%, calculated as steviol equivalents. In the beginning of 2016, Health Canada approved the use of rebaudioside M for use as a high-intensity sweetener under the same conditions as the previously approved steviol glycosides.

In Canada, the specifications are those set out for steviol glycosides in the Food Chemicals Codex or by JECFA, which indicate that steviol glycoside preparations must contain no less than 95% steviol glycosides on a dry-weight basis, must be met.

Permissions for use of SG are set out in the List of Permitted Sweeteners which covers authorized food additives that are used to impart a sweet taste to a food. It is incorporated by reference in the Marketing Authorization for Food Additives That May Be Used as Sweeteners.

Asia
Steviol glycosides are permitted as a food additive (sweetening agent) in several Asian countries including Singapore, Japan, South Korea, China, Malaysia, Indonesia, and Taiwan.

Singapore

[Regulation 18(3) and (4)], Thirteenth Schedule Permitted Sweetening Agents permits Steviol Glycosides (as steviol) at a range of MPLs, in selected Foods.

Japan
The Ministry of Health and Welfare in Japan has approved stevia extracts, α-glucosyltransferase-treated stevia, powdered stevia, and stevia extract (Japan Food Chemical Research Foundation, 2014). Purified stevioside and stevia rebaudiana leaf extracts are permitted for general use in a variety of foods and beverages including pickling gum, pickles, dried seafood, meat, fish, soy sauce, bean pastes, sugarless chewing gums, juices, cola, table-top sweeteners, and ice cream in Japan.

[10] https://www.ecfr.gov/current/title-21/part-182#p-182.1(b)

India
The Food Safety and Standards Authority of India approved the use steviol glycosides as a sweetener in eleven food and beverage categories on 20 August 2014. This included dairy based flavoured drinks, fruit nectars, non-carbonated water-based non-alcoholic beverages, carbonated water, and soft drink concentrates at a maximum level of 200mg/kg steviol equivalents.

Central/South America
In Brazil, Argentina, Paraguay, Uruguay, Mexico, Peru, and Colombia stevioside, *S. rebaudiana* leaves, and highly refined extracts are permitted for use as low-calorie sweeteners.

Other Jurisdictions[11]
Steviol glycosides are also permitted in the following countries Nigeria, Israel, Russia, Switzerland, Turkey and Ukraine.

[11] A1132 CFS p5

7 Substances added to Food – food additive

(As per section 3.3.1 [Food additives] of the Application Handbook as at 1 July 2024)

7.1 Technical Information on the food additive

(As per section 3.3.1 A of the Application Handbook as at 1 July 2024)

7.1.1 Nature and technological purpose of the food additive

(As per section 3.3.1 A.1 of the Application Handbook as at 1 July 2024)

Stevia is a plant-based sweetener extracted from the leaves of *Stevia rebaudiana*. The sweetness of *S. rebaudiana* comes from diterpene glycosides, commonly known as steviol glycosides. SG would be used in hotplate products to perform the function of an intense sweetener.

SG are currently used widely in the Australian and international food industry as an intense sweetener to replace the use of sugar partially or completely in product formulations.

The flavour and sweetness qualities of SG, coupled with their high stability, mean they are capable of wide use and can function as multi-purpose, low-calorie sweeteners.

Commercial stevia sweeteners generally contain ≥95% steviol glycosides (dry weight basis), where the steviol glycosides are defined as rebaudioside A, rebaudioside B, rebaudioside C, rubusoside, stevioside, steviolbioside, dulcoside A, rebaudioside D, rebaudioside F, rebaudioside M. In most commercial sweeteners where the extract is produced via fermentation, ratios would be different.

Suitability of SG for use in hotplate products

After reviewing the current range of permitted sweeteners listed in Schedule 15 of the Code for [6.4] flour products (including noodles and pasta), the Applicant was unable to find a suitable option for the unique formulation and process used in the manufacture of hotplate products outside of SG's.

During the product development process conducted by the Applicant, several sweetener options were identified and trialed in the manufacturing environment. Consumer acceptability and shelf-life testing was also carried out.

Commercial trials and extensive sensory evaluation works demonstrate the use of SG in hotplate products is deemed the most acceptable option when developing a range of sweet crumpets and for sugar reduction in the pancake and pikelet range. All other sugar substitutes investigated and trialed resulted in a combination of failed organoleptic results and unacceptable processing performance issues.

Permitted sweeteners currently listed in the Code like Aspartame are not heat stable and/or considered natural which is not in line with customer expectations.

During the manufacture of crumpets, a release agent is not used due to the 'pot' setup in operation. Not using a release agent means there are very limited formulation options when designing for a sweet crumpet due to the potential of batter sticking in the pots and not releasing. The Applicant trialed adding sucrose and sugar alcohols, but experienced sticking in the pots and deemed these options as unacceptable.

SG are both stable to heating and hydrolysis and seen as a more natural additive when compared to other sweetener options in the market.

For the pikelet and pancake range the Applicant is looking to provide consumers with a healthier range of snacking products by reducing the overall amount of sugar added in formulation with extended flavour range. Again, SG performed best in laboratory trials allowing a reduction in the overall amount of sucrose added while maintaining product sensory attributes and delivery on our customer expectations.

The MPL requested for SG has been determined through the product development process as delivering both the product technical and sensory objectives.

A more detailed report is provided separately as Confidential Commercial Information (CCI).

Heat-stability of steviol glycosides

The Applicant notes that most of the foods for which FSANZ has approved the addition of SG are unlikely to be heated to high temperatures.

In the Risk and technical assessment – Application A1273, FSANZ reported that:

General stability of steviol glycosides in products

JECFA concluded at its 68th meeting in 2007 that steviol glycosides are sufficiently thermally and hydrolytically stable for use in foods, including acidic beverages, under normal conditions of processing and storage (JECFA, 2007). Study results made available to JECFA for the 82nd meeting supported that the stability results can be extended to include steviol glycoside extract preparations containing higher levels of new glycosides added to the definition and appearing in commercial products, mainly rebaudioside D and rebaudioside M (FAO 2017).[12]

The Applicant notes that SG are approved for use in 'Fancy breads' at level of 160mg/kg. Such bread products are baked to a higher temperature for a longer period than hotplate products. Refer to Table 8.

Table 8: Baking/cooking temperatures and times

Product	Baking/cooking temp	Baking/cooking time
Crumpets	180^{0}C (temp of the 'pot') Core temp ~ 90^{0}C	3.0 minutes
Pikelets/Pancakes	180^{0}C (temp of the hotplate) Core temp ~ 90^{0}C	3.5 minutes
Fancy bread	190-200^{0}C Core temp ~ 95^{0}C	15-20 mins
	Note: the temperature and baking time depend on the type of bread and the kind of oven, this is an example.	

[12] A1273 Call for Submissions, November 2023, accessed 30/12/2024

The Applicant does not have specific information on what are the potential breakdown products, and what reactions might occur in hotplate products during the cooking process. The examples in Table 8 shows the hotplate products undergo a much shorter cooking time than fancy breads and would reach a lower core temperature. The Applicant proposes that prior approval for fancy breads is sufficient evidence of safety for the purpose of this application.

During the product development trials for both crumpets and pikelets/pancakes the Applicant did not identify any losses or changes to the functions of SG that impacted the organoleptic properties and appearance of these products due to the manufacturing step of heating the batter containing steviol glycosides on a hotplate.

7.1.2 Information to enable identification of the additive

(As per section 3.3.1 A.2 of the Application Handbook as at 1 July 2024)

JECFA has defined steviol glycosides as product obtained from the leaves of *Stevia rebaudiana* (Bertoni) (JECFA, 2010).

Steviol glycosides have the food additive number INS 960 (JECFA, 2005).

This application does not seek to introduce new types of stevia or modify or extend the specifications for the different types of stevia that may be used in food in Schedule 3.

Synonyms, Trade Names, and Abbreviations
SG are referred to using several other common and brand names including stevia, rebiana, rebaudioside A, Truvia™.

7.1.3 Information on the chemical and physical properties of the additive

(As per section 3.3.1 A.3 of the Application Handbook as at 1 July 2024)

All forms of stevia used in food must meet relevant chemical and physical specifications included in Schedule 3 of the Food Standards Code.

Food grade specifications for SG, finalised by JECFA, require not less than 95% of the total preparation to be comprised of ten named steviol glycosides, on a dried weight basis (JECFA, 2023, Appendix 2).

Preparations of steviol glycosides are white or light-yellow powders that are either odourless or have a slight odour. Steviol glycoside powder preparations are freely soluble in water and ethanol.

Steviol glycoside solutions have a pH between 4.5 – 7.0 (1 in 100 solution) and have sweetness profiles that range between 200-300-fold (compared to sucrose) depending on the particular steviol glycosides present in a JECFA-defined 95% pure preparation and the testing methodology details.

The heat stability of SG is discussed in Section 7.1.1.

7.1.4 Information on the impurity profile

(As per section 3.3.1 A.4 of the Application Handbook as at 1 July 2024)

All forms of stevia used in food must meet relevant purity specifications included in Schedule 3 of the Food Standards Code.

The major component of high purity steviol glycoside preparations that adhere to JECFA specifications (>95% purity) are one or a mixture of steviol glycosides. According to JECFA specifications, the impurities representing the other 5% of the material should not include more the 1% total ash, nor should residual methanol or ethanol be present at greater than 200ppm and 5,000 ppm, respectively. In addition, the specifications state that arsenic and lead levels should not exceed 1 ppm in the high purity steviol glycoside preparations (JECFA, 2023, Appendix 2).

This application seeks only to extend the use of permitted forms of SG to a new product category. As such, it is not necessary to consider the impurity profile of any new forms of SG.

7.1.5 Manufacturing Process

(As per section 3.3.1 A.5 of the Application Handbook as at 1 July 2024)

According to the International Stevia Council, to date there are four (4) known and approved production technologies to produce steviol glycosides: extraction from the stevia plant, bioconversion of stevia extract, glucosylation of stevia extract, and fermentation.[13]

1) Production by Extraction

SG from the stevia plant is produced from the crushed leaves of the stevia plant and then steeped into hot water. The liquid extract is then filtered and separated from the leaves. The high purity plant extracts are further purified with water and/or food grade alcohol (which, if used, is later removed).

2) Production by Bioconversion

Bioconversion starts with steviol glycosides extracted from the stevia leaf that are then converted to different targeted steviol glycosides, such as Reb M and Reb D or other unique glycosides with the use of enzymes – enzymes are derived from genetically modified micro-organisms. All enzymes or micro-organisms are removed from the final product, leaving purified steviol glycosides.

Bioconversion mimics the maturation process which naturally occurs within the stevia plant.

3) Production by Glucosylation

Glucosylation starts with steviol glycosides extracted from the stevia leaf. Glucose units are added using enzyme(s), producing a combination of steviol glycosides. Enzymes may or may not be derived from genetically modified micro-organisms. Some of these steviol glycosides may or may not be found in the stevia leaf.

[13] https://internationalsteviacouncil.org/about-stevia/how-stevia-is-made/#:~:text=Production%20by%20Extraction,and%20separated%20from%20the%20leaves.

4) Production by Fermentation

Fermentation starts with a genetically modified micro-organism that converts sugars into steviol glycosides like Reb M and Reb D or other unique glycosides. Fermentation mimics the process which occurs within the stevia plant in nature.

Manufacturers use the same basic steps to extract steviol glycosides from the leaves of the stevia plant, although there is some variation in the later stages of purification and separation of glycosides.

The process generally involves:

- Extraction from the leaves by dissolving the steviol glycosides in warm/hot water in a batch system, 3 – 5 times, or by a continuous reverse flow system
- Flocculation and precipitation of suspended matter
- Filtration
- Concentration by vacuum assisted evaporation
- Adsorption (and release by alcohol) in a resin exchange process
- Ion-exchange purification
- Further filtration and concentration, and
- Spray drying or crystallisation.

7.1.6 Specification for identity and purity

(As per section 3.3.1 A.6 of the Application Handbook as at 1 July 2024)

Specifications for permitted food additives are set out in Schedule 3 of the Code. They are either covered by primary sources (section S3—2), secondary sources (section S3—3) or individual specifications, section S3—5 to section S3-51.

Schedule 3 – Identity and purity contains primary sources of specifications in section S3—2. All the three primary sources have specification monographs for steviol glycosides as set out in Table 9.

SG used by the Applicant will comply with the specifications accepted under the Code.

Table 9: Code Specifications for SG

Source	Code reference	Code date	SG covered
JECFA (Joint FAO/WHO Expert Committee on Food Additives) Combined Compendium of Food Additive Specifications	subparagraph S3—2(1)(b)	JECFA, 2023, Appendix 2	Specifications are set out in 4 Annexes describing steviol glycosides based on the method of manufacture. **Annex 1** - Steviol glycosides from Stevia rebaudiana Bertoni: extraction of the leaves of Stevia rebaudiana Bertoni. **Annex 2** - Steviol glycosides from fermentation: a process in which a genetically modified microorganism is used to produce specific steviol glycosides. **Annex 3** - Enzyme modified steviol glycosides: a process in which steviol glycosides that have been extracted from the leaves of Stevia rebaudiana Bertoni undergo enzymatic conversion of major steviol glycosides to minor ones. **Annex 4** - Enzyme modified glucosylated steviol glycosides: a process in which steviol glycosides that have been extracted from the leaves of Stevia rebaudiana Bertoni undergo enzyme catalyzed reactions to add glucose units to the steviol glycosides via α-(1-4) linkages.
Food Chemicals Codex (FCC)	subparagraph S3—2(1)(c)	13th edition, 2022	
Commission Regulation (EU) No 231/2012.	subparagraph S3—2(1)(d)	No 231/2012 of 9 March 2012	Covers 11 SG
The Code Specification for steviol glycosides produced by enzymatic conversion.	S3-35		prescribed rebaudiosides are: (a) rebaudioside D; (b) rebaudioside M; and (c) rebaudioside AM.
The Code Specification for steviol glycosides from fermentation	S3-39		This specification relates to a steviol glycosides preparation that: (a) is obtained from fermentation; (b) is not obtained from the leaves of the Stevia rebaudiana Bertoni plant; and

Source	Code reference	Code date	SG covered
			(c) contains steviol glycosides that are only derived from one of the following: (i) Saccharomyces cerevisiae strain CD15407 containing novel genes for the production of steviol glycosides; (ii) Saccharomyces cerevisiae strain Y63348 containing novel genes for the production of steviol glycosides; (iii) Yarrowia lipolytica strain VRM0014 containing novel genes for the production of steviol glycosides.

This Application does not seek to introduce any forms of SG which are not already permitted for use and subject to specifications identified in Schedule 3.

Commercial stevia sweeteners proposed to be used by the Applicant only include those SG that have already been assessed and approved by FSANZ in previous applications.

Human allergenic potential

In the SD for A1273, FSANZ noted:

In previous assessments, FSANZ concluded that there is no evidence that steviol glycosides are human allergens. No new evidence challenging that conclusion was found by a literature search.[14]

7.1.7 Information for food labelling

(As per section 3.3.1 A.7 of the Application Handbook as at 1 July 2024)

SG are considered to be intense sweeteners and flavour enhancers when added to various food products. SG have been assigned the INS number of 960.

Schedule 7 – Food additive class names (for statement of ingredients) provides the list of food additive class names for labelling purposes. Schedule 8 – Food additive names and code numbers (for statement of ingredients) provides the lists of food additive names and code numbers. These lists refer to 'steviol glycosides' which has the INS code of '960'. This name applies to any steviol glycosides preparation that meets the specification and so can apply to a blend of many different individual steviol glycosides.

Substances used as food additives are required to be declared in the statement of ingredients on the label of most packaged foods. Standard 1.2.4 – Information requirements – statement of ingredients, requires food additives to be declared by their class name followed by the prescribed name, or code number in brackets.

[14] A1273 - Steviol glycosides as a food additive in Food for special medical purposes | Food Standards Australia New Zealand, accessed 30/12/2024.

SG can be listed in a statement of ingredients as either “sweetener (steviol glycosides)” or “sweetener (960)”.

7.1.8 Analytical method for detection

(As per section 3.3.1 A.8 of the Application Handbook as at 1 July 2024)

There have been analytical methods available for the detection and quantification of SG in food since preparations of SG have been commercialised and permitted as intense sweetener food additives.

Reverse-phase high-performance liquid chromatography (RP-HPLC) coupled with tandem mass spectrometry is used to detect steviol glycosides, due to its high selectivity and multianalyte capability since different sweeteners are frequently used in mixtures to achieve the desired taste, flavour, or mouthfeel (Kubica et al, 2015).

HPLC-MS based detection and quantification continues to be the recognised method for SG; however, advances have been made to increase to achieve high efficiency, simplicity, versatility, and low solvent consumption. For example, these include fast gradient UHPLC coupled with charged aerosol detection (Hollá et al., 2022), hydrophilic interaction liquid chromatography (HILIC) (Wang et al., 2022).

7.1.9 Potential additional purposes of the food additive when added to food

(As per section 3.3.1 A.9 of the Application Handbook as at 1 July 2024)

Steviol glycosides are added to food either as an intense sweetener, or to replace the sweetness normally provided by sugars. The Applicant intends to use SG in hotplate products for this purpose.

7.2 Information related to the safety of the food additive

(As per section 3.3.1 B of the Application Handbook as at 1 July 2024)

FSANZ established an ADI for steviol glycosides of 0-4 mg/kg bw/day steviol equivalents in 2008, as a part of application A540. The ADI was derived by applying a 100-fold safety factor to the No Observed Effect Level (NOEL) of 970 mg/kg bw/day (equivalent to 383 mg/kg bw/day steviol) identified in a two-year study in rats.

FSANZ has since reviewed SG several times and updated the hazard assessment for SG as part of other applications assessed from 2011 - 2024, of which the most recent is A1273 - Steviol glycosides as a food additive in Food for special medical purposes in 2024.

The Approval Report for A1273 (March 2024), stated:

FSANZ has previously assessed an extensive toxicological database on steviol glycosides, which has identified no safety concerns or a need to amend the Acceptable Daily Intake (ADI) established by FSANZ in 2008 of 0-4 mg/kg bw for steviol glycosides, expressed as steviol equivalents. This was confirmed as a part of the current assessment.

FSANZ also conducted a literature search to capture scientific studies relevant to safety that have been published since A1235. No new toxicity studies of steviol glycosides were located. [15]

The Applicant notes that in 2023 FSANZ published a refined dietary exposure assessment for steviol glycosides for the Australian and New Zealand populations, based on the results of an analytical survey of steviol glycosides in a variety of foods (FSANZ, March 2023). [16]

A hazard assessment confirmed the ADI, and the estimated dietary exposures to steviol glycosides were well below the ADI for all population groups assessed. Furthermore, no public health and safety issues were identified as a result of the risk assessment.

7.2.1 Information on the toxicokinetics and metabolism of the food additive and, if necessary, its degradation products or major metabolites

(As per section 3.3.1 B.1 of the Application Handbook as at 1 July 2024)

FSANZ have advised that for an application to extend the use of a currently permitted food additive, this section need only include the detailed reports of studies conducted since the last safety evaluation by FSANZ.

To evaluate if there are any studies in the literature investigating toxicity or safety of SG since the work on A1273 was completed, literature searches were performed using the search terms “steviol glycosides” and “steviol glycosides toxicity”. The following databases and sources were searched:

- Google Scholar
- PubMed

[15] A1273 - Steviol glycosides as a food additive in Food for special medical purposes | Food Standards Australia New Zealand, accessed 30/12/2024.
[16] Steviol glycosides (960) (intense sweetener) (stevia) | Food Standards Australia New Zealand, accessed 30/12/2024.

- Cochrane

The outcome from the above literature searches with abstracts is set out in **Appendix 3**.

In summary, the literature identified several relevant articles on steviol glycosides that have been published during 2024. These did not raise any concerns regarding the safety of steviol glycosides. The key points from each study is set out in Table 10. More information (including links) is provided in Appendix 3.

Table 10: Key Points from recent studies on SG

Title	Authors	Key points
1. A Review of Low- and No-Calorie Sweetener Safety and Weight Management Efficacy. Nutrition Today 59(6): p 261-288	Mattes et al. Nov/Dec 2024	Collectively, authoritative assessments and the primary literature support the safety of the sweeteners reviewed herein, with high concordance of safety substantiation across authoritative bodies. Taken together, and consistent with the current 2020-2025 Dietary Guidelines for Americans, the evidence indicates LNCS (Low- and no-calorie sweeteners) use is safe and may aid weight management. Note: the LNCS included stevia.
2. Scientific opinion on the extension of the authorisation of use of the food additive steviol glycosides (E 960a–d) and the modification of the acceptable daily intake (ADI) for steviol.	EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Castle, L., Sept 2024	Based on the currently available absorption, distribution, metabolism, and excretion (ADME) dataset for steviol glycosides (E 960a–d), the Panel concluded that that there is insufficient justification to increase the current ADI of 4 mg/kg bw per day, expressed as steviol equivalents. With respect to the proposed extension of use and increase of the MPLs, the Panel concluded that the calculated, conservative, dietary exposure would result in an increased exceedance of the ADI for toddlers at the 95th percentile.
3. The next generation steviol glycoside and noncaloric sweetener. Journal of Food Science, 89, 6946–6965	Okonkwo et al, September 2024	Multiple regulatory bodies, including the European Food Safety Authority (EFSA) and the U.S. FDA, have conducted safety evaluations of Reb M. These assessments indicate that Reb M is considered safe for human consumption and does not pose any adverse health effects at concentrations typically found in food products.

Title	Authors	Key points
		Toxicological studies have been conducted to assess the possible toxicity of Reb M, and the findings indicate that it is not poisonous even at large dosages and does not have significant adverse effects Moreover, Reb M is excreted unaltered in the urine and does not accumulate in the body. There have been concerns regarding the potential impact of stevia leaves or crude extracts on male fertility and male reproductive health. However, these concerns were disputed by the GRAS Notice (2019) (GRN No. 846). Considering the available information, Reb M is considered a safe Setpand nontoxic sweetener that can be used as a substitute for dietary sugars in food products.
4. In vitro Molecular Mechanisms of Anticancer Activity of Stevioside in Human Osteosarcoma Cell Lines (Sarcoma Osteogenic). Contemp Clin Dent. 2024 Jul-Sep;15(3):198-201.	Prithiksha et al, Sept 2024	Steviol glycosides have been widely used as natural noncalorie sweeteners and are the collective name of the sweet substances found naturally in the plant Stevia rebaudiana, which is commonly called Stevia. Our study aimed to analyze the anticancer activity of Stevioside in OS. The study concludes its action against bone OS cells was significant with apoptotic induction. Stevia has a wide range of health benefits as well as being a plant-based diet it has less of side effects and promoting features even by intaking it daily along with other medicines.
5. An Analysis of Toxicity of Artificial Sweeteners. (2024). Int J Pharm Sci.15(3), b63-70.	Abraham et al, July 2024	This review aims to acquire information regarding the various types of available sweeteners, their composition, their impact on human health, and a thorough comparison between natural and artificial sweeteners. This aims to assist readers in developing a healthy diet plan and making informed choices when using products that contain sweeteners.

Title	Authors	Key points
		Recent extensive research on the general and reproductive toxicity of stevioside has shown that it is safe even when consumed at high doses in the diet. Furthermore, there is no evidence or presence of genotoxicity and allergic responses associated with stevioside, as stated by Qing Yang in 2010. In the future, Stevia rebaudiana can be used as an additional component in oral care products such as mouthwash, toothpaste, chewing gum, artificial saliva, and chewable tablets.
6. Safety Assessment: Outcome of assessment of the of use of steviol glycosides produced by fermentation (Rebaudioside M) using *Saccharomyces cerevisiae*. Reference number RP1112. March 2024	Food Standards Agency (FSA) and Food Standards Scotland (FSS) Regulated Product Dossier Assessment. April 2024	Joint Expert Group on Additives, Enzymes and other regulated products (AEJEG) were asked to review the dossier and the supplementary information from the applicant. The AEJEG concluded that the new method for the production of steviol glycosides from fermentation by *Saccharomyces cerevisiae* was safe under the proposed conditions of use. The proposed uses and use levels for rebaudioside M, produced via fermentation with *Saccharomyces cerevisiae* remain the same as the already authorised food additive steviol glycosides (E 960a and E960c). The FSA and FSS therefore conclude in this assessment that the modification of the manufacturing specifications of steviol glycosides from a genetically modified production strain of Saccharomyces cerevisiae as described within this application would not pose a risk to health. Therefore, there were no concerns over safety of the proposed process.
7. Comparison of a Daily Steviol Glycoside Beverage vs. a Sucrose Beverage for Four Weeks on Gut Microflora in Healthy Adults. Journal of nutrition, 2024	Kwok et al, March 2024	Daily consumption of a beverage sweetened with 25% of the ADI of stevia for 4 weeks had no significant *effects on the HGM, fecal SCFA or fasting cardiometabolic measures, compared to daily consumption of a beverage sweetened with 30g sucrose.*

Title	Authors	Key points
8. Consumption of the Non-Nutritive Sweetener Stevia for 12 Weeks Does Not Alter the Composition of the Human Gut MicrobiotaNutrients, 2024, 16(2)	Singh et al, March 2024	The use of non-nutritive sweeteners (NNSs) as an alternative to caloric sugars has increased in recent years. Stevia is an NNS that has demonstrated beneficial effects on appetite and energy intake. However, the impact on the gut microbiota is not well understood. However, large-scale changes in the gut microbiota were not apparent in this study, and, therefore, our data suggest that stevia does not significantly impact the gut microbiota.
9. Acute and two-week effects of neotame, stevia rebaudioside M and sucrose-sweetened biscuits on postprandial appetite and endocrine response in adults with overweight/obesity-a randomised crossover trial from the SWEET consortium. EBioMedicine. 2024 Apr;102:105005	Gibbons et al, March 2024	Sweeteners and sweetness enhancers (S&SE) are used to replace energy yielding sugars and maintain sweet taste in a wide range of products, but controversy exists about their effects on appetite and endocrine responses in reduced or no added sugar solid foods. The aim of the current study was to evaluate the acute (1 day) and repeated (two-week daily) ingestive effects of 2 S&SE vs. sucrose formulations of biscuit with fruit filling on appetite and endocrine responses in adults with overweight and obesity. In conclusion, biscuits reformulated to replace sugar using StRebM or Neotame showed no differences in appetite or endocrine responses, acutely or after a two-week exposure, but can reduce postprandial insulin and glucose response in adults with overweight or obesity.

Overall, the recent studies have demonstrated that SG and their permitted equivalents, are not mutagenic or genotoxic.

There is no basis to conclude that the proposed use of stevia in hotplate products poses any risk to any segment of the general population.

7.2.2 Information on the toxicity of the food additive and, if necessary, its degradation products and major metabolites

(As per section 3.3.1 B.2 of the Application Handbook as at 1 July 2024)

Overall, the recent studies have demonstrated that SG and their permitted equivalents, are not toxic.

There is no basis to conclude that the proposed use of stevia in hotplate products poses any risk to any segment of the general population.

7.2.3 Safety assessment reports prepared by international agencies or other national government agencies, if available

(As per section 3.3.1 B.3 of the Application Handbook as at 1 July 2024)

The safety of steviol glycosides has been assessed by a number of international scientific and regulatory bodies, including JECFA, FDA, EFSA, Codex Alimentarius, FCC, GB Standards, Japanese Regulations and Health Canada. Due to the significant interest in the use of steviol glycosides extensive testing has been carried out.

JECFA has reviewed the safety of steviol glycosides in five separate meetings and has established an ADI for steviol glycosides of 0 to 4 mg/kg body weight expressed as steviol equivalents (JECFA 2005, 2007, 2008, 2010, 2023). This ADI has been adopted by FSANZ.

UK and Scotland

In April 2024 the Food Standards Agency (FSA) and Food Standards Scotland (FSS) Safety Assessment: Outcome of assessment of the of use of steviol glycosides produced by fermentation (Rebaudioside M) using *Saccharomyces cerevisiae* was published.[17]

The Joint Expert Group on Additives, Enzymes, and other regulated products (AEJEG) were asked to review the dossier and the supplementary information from the applicant. The AEJEG concluded that the new method for the production of steviol glycosides from fermentation by *Saccharomyces cerevisiae* was safe under the proposed conditions of use. The proposed uses and use levels for rebaudioside M, produced via fermentation with *Saccharomyces cerevisiae* remain the same as the already authorised food additive steviol glycosides (E 960a and E960c).

Europe

In November 2024 EFSA Panel on Food Additives and Flavourings published a Scientific opinion on the extension of the authorisation of use of the food additive steviol glycosides (E 960a-d) and the modification of the acceptable daily intake (ADI) for steviol.

The EFSA Panel on Food Additive and Flavourings (FAF Panel) evaluated the safety of proposed changes to the currently permitted uses of the food additive steviol glycosides (E 960a–d) and of a

[17] https://www.food.gov.uk/research/research-projects/safety-assessment-rp1112-modification-of-use-of-steviol-glycosides-e-960-produced-by-fermentation, accessed 30/12/2024.

proposed modification of the current acceptable daily intake (ADI) from 4 mg/kg body weight (bw) per day to 6 or 16 mg/kg bw per day, expressed as steviol equivalents.

Currently, steviol glycosides (E 960a–d) are authorised in the EU in 32 different food categories (FCs). An extension of use was proposed for four new uses within FC 7.2 'Fine bakery wares'. In addition, an increase of the maximum permitted levels (MPLs) for FC 14.1.3 'Fruit nectars' and for three uses within FC 14.1.4 'Flavoured drinks' was requested.

Consequently, the Panel updated the exposure estimates using the protocol for assessing exposure to sweeteners, developed to consider the specificities related to consumers' exposure to this functional class of food additives. Considering the proposed extension of use and increase of the MPLs, together with the currently authorised uses (at the MPLs) of E 960a–d, the highest 95th percentiles of exposure are 4.1 and 6.9 mg/kg bw per day for infants and toddlers, respectively.

Based on the currently available absorption, distribution, metabolism, and excretion (ADME) dataset for steviol glycosides (E 960a–d), the Panel concluded that that there is insufficient justification to increase the current ADI of 4 mg/kg bw per day, expressed as steviol equivalents. With respect to the proposed extension of use and increase of the MPLs, the Panel concluded that the calculated, conservative, dietary exposure would result in an increased exceedance of the ADI for toddlers at the 95th percentile.

7.3 Information related to the dietary exposure to the food additive

(As per section 3.3.1 C of the Application Handbook as at 1 July 2024)

7.3.1 A list of the food groups or foods proposed to contain the food additive, or changes to currently permitted foods

(As per section 3.3.1 C.1 of the Application Handbook as at 1 July 2024)

A list of the hotplate flour products which are proposed to contain steviol glycosides is set out in in Table 11.

The food list is based on the food group descriptions in the table to S15—5 – 6.4 - Flour products (including noodles and pasta).

Table 11: Hotplate flour products proposed to contain the food additive

Flour Product	Steviol glycosides (mg/kg)
Crumpet	350mg/kg
Pikelet	350mg/kg
Pancake	350mg/kg

7.3.2 The maximum proposed level and/or the concentration range of the food additive for each food group or food, or the proposed changes to the currently permitted levels

(As per section 3.3.1 C.2 of the Application Handbook as at 1 July 2024)

This application seeks to amend Schedule 15 to allow steviol glycosides in flour products (6.4) to a maximum level of 350mg/kg expressed as steviol equivalents. This level has been determined through product development work conducted by the Applicant.

The proposed level is lower than the maximum permitted level for other food product categories including Low joule chutneys, low joule jams and low joule spreads (permitted up to 450 mg/kg), chocolate and cocoa products (permitted up to 550 mg/kg), sugar confectionary (permitted up to 1,100 mg/kg), and tabletop sweeteners (permitted up to the minimum level required at GMP).

7.3.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

(As per section 3.3.1 C.3 of the Application Handbook as at 1 July 2024)

Inclusion of this data is not a requirement as the foods listed in Table 11 are included in the most recent Australian and New Zealand national nutrition surveys.

7.3.4 The percentage of the food group in which the food additive is likely to be found or the percentage of the market likely to use the food additive

(As per section 3.3.1 C.4 of the Application Handbook as at 1 July 2024)

Table 12: estimated % of the food group to contain SG

Flour Product Type	Estimated % of the food group estimated to contain SG
Crumpet	5%
Pikelet	5%
Pancake	5%

7.3.5 Information relating to the use of the food additive in other countries, if applicable

(As per section 3.3.1 C.5 of the Application Handbook as at 1 July 2024)

SG are added to many different foods and food groups overseas, and at varying levels per serve. Information on SG-containing foods launched over the last 5 years is provided in Table 13.

Table 13: SG containing bakery products sold within the last 5 years worldwide.

Sub-Category	Total Sample
Sweet Biscuits/Cookies	452
Cakes, Pastries & Sweet Goods	434
Baking Ingredients & Mixes	218
Bread & Bread Products	109
Savoury Biscuits/Crackers	12
Total Sample	1225

Source: Mintel GNPD Analysis, September 2, 2024

7.3.6 For foods where consumption has changed in recent years, information on likely current food consumption

(As per section 3.3.1 C.6 of the Application Handbook as at 1 July 2024)

The Applicant has provided information on the current and expected sales of hotplate products formulated to contain SG as well as current hotplate sales to support this application. This information is provided as CCI.

8 General food labelling

(As per section 3.2.1 of the Application Handbook as at 1 July 2024)

8.1 General Information to support the proposed labelling change

(As per section 3.2.1 A of the Application Handbook as at 1 July 2024)

The Applicant is **not** proposing a change to food labelling however, the following comments are provided in relation to labelling of SG in a food product for the sake of completeness.

The existing requirements in the Code for the labelling and advertising of foods containing added SG will apply.

Declarations of SG in the statement of ingredients will enable consumers to identify and monitor their consumption of SG.

REFERENCES

Marcela Hollá, Dalibor Šatínský, František Švec, Hana Sklenářová, UHPLC coupled with charged aerosol detector for rapid separation of steviol glycosides in commercial sweeteners and extract of Stevia rebaudiana, Journal of Pharmaceutical and Biomedical Analysis, Volume 207, 2022. https://doi.org/10.1016/j.jpba.2021.114398
https://www.sciencedirect.com/science/article/abs/pii/S0731708521005094

Kubica, P., Namieśnik, J. & Wasik, A. Determination of eight artificial sweeteners and common Stevia rebaudiana glycosides in non-alcoholic and alcoholic beverages by reversed-phase liquid chromatography coupled with tandem mass spectrometry. Anal Bioanal Chem 407, 1505–1512 (2015). https://doi.org/10.1007/s00216-014-8355-x

https://link.springer.com/article/10.1007/s00216-014-8355-x

Jing Wang, Yang Zhao, Yang Yang, Xin Chen, Yu Jin, Yanxiong Ke, Separation of minor steviol glycosides using hydrophilic interaction liquid chromatography (HILIC) and off-line two-dimensional reversed-phase liquid chromatography/HILIC methods, Journal of Food Composition and Analysis, Volume 112, 2022. https://doi.org/10.1016/j.jfca.2022.104683
https://www.sciencedirect.com/science/article/abs/pii/S0889157522003015

Appendix 1 - GSFA Online Food Additive Group Details for Steviol Glycosides

https://www.fao.org/gsfaonline/groups/details.html?id=309

Appendix 2 - JECFA Monograph

Monograph 31, 2023, 96th meeting.

Appendix 3: Literature search results on SG studies for 2024

Sources: Google Scholar, PubMed, Cochrane

Timeline: January - December 2024 inclusive

Search terms: "Steviol glycosides" and "steviol glycosides toxicity"

Search results

1. Mattes, Richard D. PhD, MPH, RD; Rivera, Brianna N. PhD; Rutigliani, Giorgia; Rogers, Sarah MS; Mendoza, Ivan D. MPH; Wang, Lucheng; Beckemeier, Katheryn; Wikoff, Daniele PhD. A Review of Low- and No-Calorie Sweetener Safety and Weight Management Efficacy. Nutrition Today 59(6):p 261-288, 11/12 2024. | DOI: 10.1097/NT.0000000000000723

Abstract: Low- and no-calorie sweeteners (LNCSs) impart sweetness while providing little or no energy. Their safety and weight management efficacy remain unsettled science that leaves open questions among consumers, researchers, clinicians, and policy makers. The objective of this narrative review is to provide a critical consideration of the safety and efficacy of weight management evidence for LNCSs that have been reviewed/approved by the US Food and Drug Administration and have the highest frequency of use: acesulfame potassium, allulose, aspartame, erythritol, monk fruit, saccharin, stevia, sucralose, and xylitol. Safety assessments by the authoritative bodies for the World Health Organization, European Union, and United States were reviewed. Additionally, emerging topics of interest regarding the safety of these sweeteners commonly cited in the recent literature or highlighted in the media are discussed. Collectively, authoritative assessments and the primary literature support the safety of the sweeteners reviewed herein, with high concordance of safety substantiation across authoritative bodies. Weight management efficacy, measured by various adiposity indices in epidemiological studies, ranges from no effect to a slight positive association. Clinical trials with various mixtures of LNCSs more consistently indicate LNCS use is associated with lower adiposity indices. The latter are ascribed greater evidentiary weight, and recent application of statistical methods to better correct for potential biases in cohort studies reveals they are more consistent with the clinical trial findings. Studies that investigated individual sweeteners were limited but suggestive of differing effects or lack of sufficient data to support any formal conclusions on their efficacy for weight management. Taken together, and consistent with the current 2020-2025 Dietary Guidelines for Americans, the evidence indicates LNCS use is safe and may aid weight management.

https://journals.lww.com/nutritiontodayonline/fulltext/2024/11000/a_review_of_low__and_no_calorie_sweetener_safety.4.aspx

2. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Castle, L., Andreassen, M., Aquilina, G., Bastos, M. L., Boon, P., Fallico, B., FitzGerald, R., Frutos Fernandez, M. J., Grasl-Kraupp, B., Gundert-Remy, U., Gürtler, R., Houdeau, E., Kurek, M., Louro, H., Morales, P., Passamonti, S., Barat Baviera, J. M., Degen, G.,… Vermeiren, S. (2024). Scientific opinion on the extension of the authorisation of use of the food additive steviol glycosides (E 960a–d) and the modification of the acceptable daily intake (ADI) for steviol. EFSA Journal, 22(11), e9045. https://doi.org/10.2903/j.efsa.2024.9045

Abstract

The EFSA Panel on Food Additive and Flavourings (FAF Panel) evaluated the safety of proposed changes to the currently permitted uses of the food additive steviol glycosides (E 960a–d) and of a proposed modification of the current acceptable daily intake (ADI) from 4 mg/kg body weight (bw) per day to 6 or 16 mg/kg bw per day, expressed as steviol equivalents. Currently, steviol glycosides (E 960a–d) are authorised in the EU in 32 different food categories (FCs). An extension of use was proposed for four new uses within FC 7.2 'Fine bakery wares'. In addition, an increase of the maximum permitted levels (MPLs) for FC 14.1.3 'Fruit nectars' and for three uses within FC 14.1.4 'Flavoured drinks' was requested. Consequently, the Panel updated the exposure estimates using the protocol for assessing exposure to sweeteners, developed to consider the specificities related to consumers' exposure to this functional class of food additives. Considering the proposed extension of use and increase of the MPLs, together with the currently authorised uses (at the MPLs) of E 960a–d, the highest 95th percentiles of exposure are 4.1 and 6.9 mg/kg bw per day for infants and toddlers, respectively.

Based on the currently available absorption, distribution, metabolism, and excretion (ADME) dataset for steviol glycosides (E 960a–d), the Panel concluded that that there is insufficient justification to increase the current ADI of 4 mg/kg bw per day, expressed as steviol equivalents. With respect to the proposed extension of use and increase of the MPLs, the Panel concluded that the calculated, conservative, dietary exposure would result in an increased exceedance of the ADI for toddlers at the 95th percentile.

https://efsa.onlinelibrary.wiley.com/share/NTXJTJBQIHT38UHZPPG2?target=10.2903/j.efsa.2024.9045

3. Okonkwo, C. E., Adeyanju, A. A., Onyeaka, H., Nwonuma, C. O., Olaniran, A. F., Alejolowo, O. O., Inyinbor, A. A., Oluyori, A. P., & Zhou, C. (2024). A review on rebaudioside M: The next generation steviol glycoside and noncaloric sweetener. Journal of Food Science, 89, 6946–6965. https://doi.org/10.1111/1750-3841.17401

Abstract

So far, the use of artificial low-calorie sweeteners, like sucralose, saccharin, and so on, to replace the conventional-based sugars has not succeeded due to the long-term adverse health effects, for example, hypertension, and not well-known safety stand. In this review, we discussed the next generation SvGl (rebaudioside M [Reb M]), their biosynthetic pathway in plant, high-yield production via microbial fermentation and enzyme engineering, physicochemical properties, taste modification, kinetic metabolism, application in food and beverages, safety and toxicological evaluation, regulation and dosage recommendation, and health benefits. In stevia, the biosynthesis of stevia glycosides, especially Reb M, is derived from the bifurcation of the pathway leading to gibberellin, followed by subsequent enzymatic modification of rubusoside. Reb M is more economically produced via microbial fermentation of modified yeast Yarrowia lipolytica and enzymatic bioconversion of rebaudioside A (Reb A) or Reb E. Reb M can serve as a suitable alternative to the conventional-based sugars.

SAFETY AND TOXICOLOGICAL EVALUATION OF REBAUDIOSIDE M

Reb M, a natural sweetener derived from the stevia plant, has undergone comprehensive safety and toxicological evaluations by regulatory bodies worldwide to ensure its safety for human consumption and to assess its potential adverse health effects (EFSA, 2019; Perrier et al., 2018). Multiple regulatory bodies, including the European Food Safety Authority (EFSA) and the U.S. FDA, have conducted safety evaluations of Reb M. These assessments indicate that Reb M is considered safe for human consumption and does not pose any adverse health effects at concentrations typically found in food products. In 2015, a manufacturer requested modifications to the specifications of SvGls (E 960) from the EFSA ANS Panel, which included the addition of Reb M to the list of SvGls that can constitute up to 95% of the total content, and the elimination of the prerequisite that SvGls must have a minimum of 75% stevioside or Reb A. The applicant justified these changes by aligning them with the current use of SvGl preparations containing Reb M as a high-intensity sweetener in food and beverages, which would adhere to Commission Regulation (EU) No. 1131/2014 on SvGls. Reb M, previously identified as a minor SvGls component of the S. rebaudiana Bertoni plant by Prakash, Markosyan, et al. (2014), was included in the proposed modifications.

Commercial stevia extracts typically contain low levels of Reb M and other known steviol glycosides (Prakash & Chaturvedula, 2018). However, the applicant of this study has developed a production process that selectively isolates Reb M, resulting in SvGls preparations enriched with this compound (Prakash et al., 2013). The quality and consistency of the resulting product were assessed through tests conducted on batches of high purity Reb M and a SvGl extract containing approximately 50% Reb M. The findings indicate that the manufacturing process yields a consistent product that largely complies with EU specifications for SvGls, except for the assay value. In addition to evaluating the product's quality, the applicant also provided data on the stability of Reb M, including assessments of its bulk stability under normal and extreme storage conditions, as well as at various temperatures

and pH levels. The safety of including Reb M as alternatives to Reb A in the predominant components of SvGls was evaluated by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) in response to a request from the European Commission. Based on a thorough review of the available evidence, the EFSA ANS Panel concluded that the proposed amendment would not pose a safety concern (EFSA, 2015).

Toxicological studies have been conducted to assess the possible toxicity of Reb M, and the findings indicate that it is not poisonous even at large dosages and does not have significant adverse effects (Perrier et al., 2018; Purkayastha et al., 2016; Soejarto et al., 2019). Moreover, Reb M is excreted unaltered in the urine and does not accumulate in the body. There have been concerns regarding the potential impact of stevia leaves or crude extracts on male fertility and male reproductive health.

However, these concerns were disputed by the GRAS Notice (2019) (GRN No. 846). Considering the available information, Reb M is considered a safe and nontoxic sweetener that can be used as a substitute for dietary sugars in food products.

https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.17401

4. Prithiksha N, Priyadharshini R. In vitro Molecular Mechanisms of Anticancer Activity of Stevioside in Human Osteosarcoma Cell Lines (Sarcoma Osteogenic). Contemp Clin Dent. 2024 Jul-Sep;15(3):198-201. doi: 10.4103/ccd.ccd_429_23. Epub 2024 Sep 25. PMID: 39512298; PMCID: PMC11540208.

Abstract

Introduction:

Osteosarcoma (OS) is a rare and aggressive form of bone cancer that primarily affects the long bones of the body, such as the arms and legs. It is characterized by the uncontrolled growth of malignant cells in the bone tissue, leading to the formation of abnormal and painful bone masses.

Steviol glycosides have been widely used as natural noncalorie sweeteners and are the collective name of the sweet substances found naturally in the plant Stevia rebaudiana, which is commonly called Stevia. Our study aimed to analyze the anticancer activity of Stevioside in OS.

Materials and Methods:

Stevioside was applied to OS cells, and the levels of Bcl xL, Bcl-2, and Bax were then estimated. The results of three separate studies, each carried out in triplicate, were expressed as the mean ± standard errors of the mean (SEM). One-way ANOVA was used for statistical analysis.

Results:

The findings showed that the effect of Stevioside on sarcoma osteogenic cells with mean ± SEM as 0.74 ± 0.05, 0.69 ± 0.09, 0.46 ± 0.09 for Bcl-xL gene, 0.98 ± 0.06, 0.58 ± 0.07, 0.5 ± 0.07 for Bcl-2 gene, and 1.2 ± 0.08, 1.45 ± 0.11, 1.67 ± 0.12 for Bax gene, respectively, when treated with untreated control cells.

Conclusion:

The study concludes its action against bone OS cells was significant with apoptotic induction. Stevia has a wide range of health benefits as well as being a plant-based diet it has less of side effects and promoting features even by intaking it daily along with other medicines.

https://pmc.ncbi.nlm.nih.gov/articles/PMC11540208/

5. John Abraham, Gurshan Singh Gill, Asit Kumar, Y. C. Tripathi. An Analysis of Toxicity of Artificial Sweeteners. (2024). Int J Pharm Sci.15(3), b63-70 http://dx.doi.org/10.22376/ijpbs.2024.15.3.b63-70

Abstract: Monosaccharides and disaccharides, natural sweeteners, have some nutritional value, but artificial sweeteners have few or absence of nutritional advantages. The influence of sugar on health is a subject of debate, especially in light of increasing apprehensions over diabetes and obesity. In the 21st century, a growing number of individuals opt for low-calorie, artificially sweetened items to control their weight and blood glucose levels due to increasing health awareness. Although widely used, artificial sweeteners are controversial because of their safety and effectiveness concerns. According to reports, these medications may have adverse effects such as headaches, weight gain, and organ malfunction, which raise concerns about their prolonged usage. Conversely, sweeteners derived from natural sources, such as Stevia, are seen as being more secure. While the FDA has approved some artificial sweeteners, there is still ongoing dispute regarding their metabolic consequences. Hence, customers had to select between synthetic and organic sweeteners meticulously. The early published reviews of the "Artificial Sweeteners" were deficient in extensive long-term human investigations, mainly depended on animal research, neglected to analyze different types of sweeteners, and disregarded confounding factors such as food and lifestyle.

This review aims to acquire information regarding the various types of available sweeteners, their composition, their impact on human health, and a thorough comparison between natural and artificial sweeteners. This aims to assist readers in developing a healthy diet plan and making informed choices when using products that contain sweeteners.

Conclusion: Recent extensive research on the general and reproductive toxicity of stevioside has shown that it is safe even when consumed at high doses in the diet. Furthermore, there is no evidence or presence of genotoxicity and allergic responses associated with stevioside, as stated by Qing Yang in 2010. In the future, Stevia rebaudiana can be used as an additional component in oral care products such as mouthwash, toothpaste, chewing gum, artificial saliva, and chewable tablets.

Considering its medicinal advantages, it is a boon and particularly advantageous for those who are obese, diabetic, or have hypertension. The research given in this review paper indicates that using artificial sweeteners is linked to many negative health consequences. Regular intake of NAS (noncaloric artificial sweeteners) is associated with exacerbating glucose intolerance, which raises concerns over their effects on metabolic health. Moreover, the possible hazard of cardiovascular ailments linked to artificial sweeteners emphasizes the necessity for additional investigation in this field to determine causation. The analysis also underscores the hazardous capacity of several frequently employed artificial sweeteners, underscoring the significance of cautious deliberation while using these drugs. The correlation between artificial sweeteners and colon cancer elicits apprehension about their enduring safety. Furthermore, the adverse impacts of artificial sweeteners on the immune system and the potential correlation with bladder cancer underscore the significance of closely monitoring and controlling their inclusion in food items. This review paper emphasizes the importance of being cautious and conducting further research on the safety of artificial sweeteners despite their promotion as sugar alternatives for those seeking to reduce calorie consumption or control diabetes.

Reference provided

6. Safety Assessment RP1112. Modification of use of Steviol Glycosides (E960) produced by Fermentation, April 2024

Food Standards Agency (FSA) and Food Standards Scotland (FSS) Regulated Product Dossier Assessment

Summary: An application was submitted to the Food Standards Agency (FSA) and Food Standards Scotland (FSS) in May 2021 from Amyris Inc ("the Applicant") for changes of the existing production method of steviol glycosides (formerly E 960) to include fermentation by Saccharomyces cerevisiae.

The production method uses a genetically modified strain of S. cerevisiae to convert sugar into rebaudioside M (reb M) at a final purity of no less than 95% reb M (anhydrous).

To support the FSA and FSS in evaluating the dossier the Joint Expert Group on Additives, Enzymes and other regulated products (AEJEG) were asked to review the dossier and the supplementary information from the applicant. The AEJEG concluded that the new method for the production of steviol glycosides from fermentation by *Saccharomyces cerevisiae* was safe under the proposed conditions of use. The proposed uses and use levels for rebaudioside M, produced via fermentation with *Saccharomyces cerevisiae* remain the same as the already authorised food additive steviol glycosides (E 960a and E960c).

The AEJEG requested that the Applicant perform an exposure assessment for the minor impurity kaurenoic acid. The Applicant stated that exposure to kaurenoic acid from steviol glycosides manufactured by fermentation (Reb M), can be effectively estimated using recent estimates of anticipated steviol glycoside exposure conducted by EFSA.

The AEJEG requested further work to be performed regarding the exposure assessment of kaurenoic acid (discussed in 'Safety Assessment of Kaurenoic Acid' later in this document). This was provided by the Applicant and the AEJEG was overall satisfied with the exposure assessment for kaurenoic acid.

Conclusions: To support the FSA and FSS in evaluating the dossier, the Additives and Enzymes Joint Expert Group (AEJEG) were asked to review the dossier submitted by the Applicant and the subsequent additional information requested and advise the FSA and FSS. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) also reviewed the AEJEG advice and agreed with the conclusions of the AEJEG. The FSA and FSS agreed on the conclusions of the AEJEG in that the proposed change in the steviol glycoside specification to include a production method using Saccharomyces cerevisiae to convert sugar into Reb M via fermentation is safe under the proposed conditions of use and at the anticipated levels of intake. The AEJEG advised the FSA and FSS that sufficient information had been provided to allow for an evaluation of the proposal for modification of the manufacturing specifications of steviol glycosides from a genetically modified production strain of Saccharomyces cerevisiae, there were no concerns over safety of the proposed process. Regarding the impurity kaurenoic acid which may be present at a maximum concentration of 300 mg/kg in steviol glycosides from fermentation. The FSA and FSS, on advice from the AEJEG, were satisfied by the information received from the Applicant regarding the derivation of a safe human exposure level for kaurenoic acid. The FSA and FSS therefore conclude in this assessment that the modification of the manufacturing specifications of steviol glycosides from a genetically modified production strain of Saccharomyces cerevisiae as described within this

application would not pose a risk to health. Therefore, there were no concerns over safety of the proposed process.

https://www.food.gov.uk/research/research-projects/safety-assessment-rp1112-modification-of-use-of-steviol-glycosides-e-960-produced-by-fermentation

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KY: *Stevia; *beverage; *intestine; *microflora; Adult; Aged; Anthropometry; Article; Blood pressure; Controlled study; Double blind procedure; Fasting; Female; Food intake; Glucose blood level; Human; Liquid chromatography-mass spectrometry; Male; Normal human; Parallel design; Phylum; Randomized controlled trial; Shotgun sequencing; Sugar-sweetened beverage; Ultra performance liquid chromatography

AB: BACKGROUND: Recent studies suggest that some non-nutritive sweeteners (NNS) have deleterious effects on the human gut microbiome (HGM). The effect of steviol glycosides on the HGM has not been well studied.

OBJECTIVE: We aimed to evaluate the effects of stevia- versus sucrose-sweetened beverages on the HGM and fecal short-chain-fatty-acid (SCFA) profiles.

METHODS: Using a randomized, double blinded, parallel design study, n=59 healthy adults (female/male n=36/23, aged 31±9 years, BMI: 22.6±1.7 kg/m2) consumed 16oz of a beverage containing either 25% of the Acceptable-Daily-Intake (ADI) of stevia or 30g sucrose daily for 4 weeks followed by a 4-week washout. At weeks 0 (baseline), 4 and 8 the HGM was characterized via shotgun sequencing, fecal SCFA concentrations were measured using ultra-high performance liquid chromatography-tandem mass spectrometry and anthropometric measurements, fasting serum glucose, insulin and lipids, blood pressure, pulse and 3-day diet records were obtained. RESULTS: There were no significant differences in the HGM or fecal SCFA between the stevia and sucrose groups at baseline ($P > 0.05$). At week 4 (post-intervention) there were no significant differences in the HGM at the phylum, family, genus or species level between the stevia and sucrose groups and no significant differences in fecal SCFA. At week 4, BMI had increased by 0.3 kg/m2 ($P = 0.013$) in sucrose versus stevia but all other anthropometric and cardiometabolic measures and food intake did not differ significantly ($P > 0.05$). At week 8 (post-washout) there were no significant differences in the HGM, fecal SFCA, or any anthropometric or cardiometabolic measure between the stevia and sucrose groups ($P > 0.05$).

CONCLUSIONS: Daily consumption of a beverage sweetened with 25% of the ADI of stevia for 4 weeks had no significant effects on the HGM, fecal SCFA or fasting cardiometabolic measures, compared to daily consumption of a beverage sweetened with 30g sucrose.

US: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-02668043/full

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Abstract: The use of non-nutritive sweeteners (NNSs) as an alternative to caloric sugars has increased in recent years. Stevia is an NNS that has demonstrated beneficial effects on appetite and energy intake. However, the impact on the gut microbiota is not well understood. Therefore, we investigated how regular consumption of stevia, for up to 12 weeks, impacts the human gut microbiota. Healthy subjects with a normal body mass index participated in our study; the stevia group (n = 14) was asked to consume five drops of stevia twice daily, compared to control participants (n = 13). Faecal samples collected before and after treatment were analysed by 16S rRNA gene sequencing. Stevia did not cause significant changes in the alpha or beta diversity when compared to the control groups. When the relative abundances of taxa were investigated, no clear differences were detected. Conversely, a random forest analysis correctly associated the gut microbiome with the control and stevia groups with an average of 75% accuracy, suggesting that there are intrinsic patterns that could discriminate between control and stevia use. However, large-scale changes in the gut microbiota were not apparent in this study, and, therefore, our data suggest that stevia does not significantly impact the gut microbiota.

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Summary

Background

Sweeteners and sweetness enhancers (S&SE) are used to replace energy yielding sugars and maintain sweet taste in a wide range of products, but controversy exists about their effects on appetite and endocrine responses in reduced or no added sugar solid foods. The aim of the current study was to evaluate the acute (1 day) and repeated (two-week daily) ingestive effects of 2 S&SE vs. sucrose formulations of biscuit with fruit filling on appetite and endocrine responses in adults with overweight and obesity.

Methods

In a randomised crossover trial, 53 healthy adults (33 female, 20 male) with overweight/obesity in England and France consumed biscuits with fruit filling containing 1) sucrose, or reformulated with either 2) Stevia Rebaudioside M (StRebM) or 3) Neotame daily during three, two-week intervention periods with a two-week washout. The primary outcome was composite appetite score defined as [desire to eat + hunger + (100 − fullness) + prospective consumption]/4.

Findings

Each formulation elicited a similar reduction in appetite sensations (3-h postprandial net iAUC). Postprandial insulin (2-h iAUC) was lower after Neotame (95% CI (0.093, 0.166); $p < 0.001$; $d = -0.71$) and StRebM (95% CI (0.133, 0.205); $p < 0.001$; $d = -1.01$) compared to sucrose, and glucose was lower after StRebM (95% CI (0.023, 0.171); $p < 0.05$; $d = -0.39$) but not after Neotame (95% CI (−0.007, 0.145); $p = 0.074$; $d = -0.25$) compared to sucrose. There were no differences between S&SE or sucrose formulations on ghrelin, glucagon-like peptide 1 or pancreatic polypeptide iAUCs. No clinically meaningful differences between acute vs. two-weeks of daily consumption were found.

Interpretation

In conclusion, biscuits reformulated to replace sugar using StRebM or Neotame showed no differences in appetite or endocrine responses, acutely or after a two-week exposure, but can reduce postprandial insulin and glucose response in adults with overweight or obesity.

https://pmc.ncbi.nlm.nih.gov/articles/PMC11026940/#:~:text=In%20conclusion%2C%20biscuits%20reformulated%20to,adults%20with%20overweight%20or%20obesity.

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An Analysis of Toxicity of Artificial Sweeteners

[1] John Abraham, [2] Gurshan Singh Gill, [3*] Asit Kumar, [4] Y. C. Tripathi,

[1] Assistant Professor, Department of Family Medicine, St. Johns National Academy of Health Sciences, Bangalore, India- 560034
[2] Assistant Professor, Department of Community Medicine, Christian Medical College and Hospital, Baba Farid University of Health Sciences, Ludhiana, India
[3] Assistant Professor, Environmental Science Department, Rajdhani College, University of Delhi, India.
[4] Chemistry and Bioprospecting Division, Forest Research Institute, PO New Forest, Dehradun-248006, India

Abstract: Monosaccharides and disaccharides, natural sweeteners, have some nutritional value, but artificial sweeteners have few or absence of nutritional advantages. The influence of sugar on health is a subject of debate, especially in light of increasing apprehensions over diabetes and obesity. In the 21st century, a growing number of individuals opt for low-calorie, artificially sweetened items to control their weight and blood glucose levels due to increasing health awareness. Although widely used, artificial sweeteners are controversial because of their safety and effectiveness concerns. According to reports, these medications may have adverse effects such as headaches, weight gain, and organ malfunction, which raises concerns about their prolonged usage. Conversely, sweeteners derived from natural sources, such as stevia, are seen as being more secure. While the FDA has approved some artificial sweeteners, there is still ongoing dispute regarding their metabolic consequences. Hence, customers had to select between synthetic and organic sweeteners meticulously. The early published reviews of the "Artificial Sweeteners" were deficient in extensive long-term human investigations, mainly depended on animal research, neglected to analyze different types of sweeteners, and disregarded confounding factors such as food and lifestyle. This review aims to acquire information regarding the various types of available sweeteners, their composition, their impact on human health, and a thorough comparison between natural and artificial sweeteners. This review aims to assist readers in developing a healthy diet plan and making informed choices when using products that contain sweeteners.

Keywords: Artificial Sweeteners, Aspartame, Neotame, Natural sweetener, Stevia

***Corresponding Author**

Asit Kumar , Assistant Professor, Environmental Science Department, Rajdhani College, University of Delhi, India.

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1 INTRODUCTION

The sweet taste is usually considered the most enjoyable sensation, as humans inherently prefer sweetness. This preference for sweetness motivated our evolutionary primate ancestors to select meals high in energy, potentially serving as a safeguard against starving by allowing the body to store excess calories. Sweeteners are culinary additives utilized to enhance the taste of common food items. Natural sweeteners have a sweet taste and provide some nutritional benefits. The primary component of natural sweeteners is either monosaccharides or disaccharides[1]. Conversely, artificial sweeteners are substances that possess minimal or negligible nutritional worth. However, the significance of sugar in our diet and its impact on our lives continues to be a highly debated subject. In 2007, diabetes accounted for 68% of global fatalities, with diabetic people being 2-4 times more prone to experiencing a heart attack (Centers for Disease Control, 2007)[2]. In the 21st century, there has been a significant shift globally, with people increasingly aware of their nutrition and health. Many individuals are adopting dietary restrictions or calorie reduction to manage chronic illnesses. Each year, several new diets are being introduced to reduce the excessive weight of millions of individuals. An effective and simple approach to decreasing calorie intake is replacing high-calorie drinks and food products with artificially sweetened options with fewer calories[3]. Artificial sweeteners have been a growing debate in food, health, and nutrition. Consumers frequently encounter many conflicting perspectives and data concerning the safety and effectiveness of artificial sweeteners. Typically, those who are mindful of their diet and are obese or have diabetes utilize low-calorie and sugar-free items to reduce calorie intake and regulate blood glucose levels. Registered Dietitians (RDs) are responsible for providing patients with correct and reliable information on using sweeteners in various food products[4]. The proliferation of contradictory data has generated several inquiries and uncertainties regarding the utilization and safety of artificial and non-nutritive sweeteners in everyday existence. Using artificial sweeteners can lead to several adverse effects, including migraines or headaches, skin eruptions, muscular dysfunction, depression, weight gain, liver and kidney impacts, impaired vision, multiple sclerosis, and symptoms similar to fibromyalgia. According to some experts, taking artificial sweeteners might lead to notable alterations in hunger, which can cause weight gain due to consuming more calories[5]. In addition, several healthcare practitioners and scientists propose that further investigation is necessary to authorize the regular consumption of artificial sweeteners over an extended period. Despite the lack of peer-reviewed scientific studies supporting them, the majority of websites and sources, users mostly rely on basic internet searches to obtain information and begin using artificial sweeteners without seeking professional advice. Natural sweeteners are considered harmless in comparison to artificial sweeteners due to their extraction from plants. Stevia, a natural sweetener, has gained significant popularity in recent years. It is promoted as an all-natural sweetener and a substitute for artificial sweeteners[6]. The food business employs a variety of artificial sweeteners with low-calorie content, as opposed to high-calorie sugar. The U.S. Food and Drug Administration has approved using aspartame, acesulfamek, neotame, cyclamate, and alitame based on their acceptable daily intake (ADI) value[7]. However, the physiological and metabolic implications of these sweeteners' breakdown products remain contentious to the present day. However, unusual sugars are monosaccharides that do not metabolize in our body and, therefore, have no known health impacts. Despite this, they have the same sweet taste and bulk characteristics as regular sugar. Rare sugars do not have an "Acceptable Daily Intake" (ADI) value and are mostly produced through the utilization of bioreactors[8]. Consequently, despite their great demand, rare sugars cannot be manufactured in the needed amounts. Artificial sweeteners have been shown to induce abnormalities in the shape and size of sperm, including the presence of a ring shape at the end of the sperm tail, coiled sperm, a shortened tail, and the appearance of an intracellular fluid in the mid-piece[9]. Consumers are frequently bombarded with many conflicting viewpoints and data on the safety and effectiveness of sweeteners. The usage of artificial sweeteners may lead to migraines or headaches, skin eruptions, muscular dysfunction, depression, weight gain, cancer, liver and kidney damage, multiple sclerosis, and impaired eyesight[10]. However, it is worth noting that natural sweeteners such as stevia and its derivatives are considered harmless and do not pose any health risks. Therefore, consumers must exercise caution while selecting sweeteners.

2 ARTIFICIAL SWEETENERS

Artificial sweeteners may be categorized into two groups based on their energy content. The primary types of nutritive sweeteners are predominantly mono-saccharides, polyols such as xylitol and sorbitol, and disaccharide polyols including lactitol and maltitol[11]. These substances' energy content is equivalent to sucrose's. The non-nutritive sweeteners consist of molecules from many chemical families that possess a sweet flavor and are significantly sweeter than sucrose, ranging from 30 to 13,000 times.

2.1 Aspartame (APM)

Aspartame (APM) is a compound made up of the methyl ester of the dipeptide L-L-aspartyl-L-phenylalanine. It has a molecular weight of 294.3 and provides 4 kcal/g of energy, according to the Food and Drug Administration (2006)[12]. Fry (1999) states that APM is the most widely utilized artificial sweetener globally. It has been discovered that it is 200 times more sweet than sucrose, and for the past 30 years, it has been used as a food ingredient in many culinary items. Research on aspartame indicates alterations in body weight, weight gain, and food intake. The observed consequences are not a result of the harmful characteristics of neotame but rather owing to the lack of taste appeal in meals that include this sweetener[13]. Consequently, rats will reduce their daily food consumption, leading to a sustained fall in body weight and reduced weight increase. No negative effects were observed in definitive safety studies regarding using neotame. Clinical observations, physical examinations, water consumption, and clinical pathology evaluations revealed no adverse findings[14]. There were no instances of illness, death, organ toxicity, or any macroscopic or microscopic postmortem findings. Aspartame has been linked to the development of several clinical diseases, such as hepatotoxicity, nephrotoxicity, neurotransmitter imbalance, and cognitive impairments. It was noted that males

were more susceptible than females in several instances. An elevated concentration of the naturally occurring essential amino acid phenylalanine poses a health risk to those with phenylketonuria (PKU), a rare hereditary disorder[15]. Patients with phenylketonuria should avoid consuming aspartame. Aspartame may be a primary factor in causing changes in behavior, such as impulsive conduct, less patience, decreased physical activity, and impaired neuromuscular coordination. Consuming aspartame during pregnancy may have detrimental effects on the developing baby. The study found that aspartame treatment in rats resulted in a decrease in the average weights of the placenta and the mother-fetus, as well as a reduction in umbilical cord length and certain measurements of the nuclei of fetal hepatocytes[16]. These changes were well-documented in the majority of kariometric parameters of the hepatocytes. Exposure to aspartame during pregnancy may impact the ability to understand and navigate space, as well as the regulation of glucose levels in mice, especially in males. After consuming aspartame, individuals saw temporary increases in body weight, blood pressure, and glucose and lipids levels in the blood. Additionally, there was a temporary decrease in plasma urea. The impact of consuming aspartame on glucose regulation was extensively recorded ten years prior. At a concentration, aspartame can modify the typical collection of antioxidant enzymes in various organs such as the liver and kidney[17]. Prolonged ingestion of aspartame can also result in oxidative stress in red blood cells and other blood cells. The administration of aspartame was identified as a primary factor contributing to oxidative stress in immunological organs, including the spleen, thymus, lymph nodes, and bone marrow of rats with a folate deficiency[18]. Generating free radicals in highly vulnerable tissues may weaken immune responses and increase infection susceptibility. Aspartame has been identified as a possible carcinogen in several animal models. Nevertheless, there needed to be more substantial evidence from the epidemiological evaluation.

2.2 Acesulfame-K, also known as Ace-K:

Ace-K is said to be 200 times sweeter than sugar, and its level of sweetness is comparable to that of aspartame. Ace-K is noncaloric since the body does not metabolize it, and approximately 95% of the sweetener is excreted without being used by the body[19]. The chemical formula of the compound is 6-methyl-1-2-3-oxathiazine-4(3H)-1-2-2-dioxide. In 1967, German scientists Clauss and Jensen discovered this delicious chemical while researching at the Nutrinova Lab. Ace-K was granted FDA approval for use in the beverage industry in 1998. The FDA authorized its widespread use and consumption in 2003, except for chicken and other meals. The acceptable daily dose (ADI) of Ace-K is 15 milligrams per kilogram of body weight per day[20]. The substance is commercially accessible under the brand names Sunett and Sweet One. It is used in various food items such as frozen desserts, breath mints, sweets, baked goods, cough drops, and drinks. Acesulfame-K, an artificial sweetener, has been subject to close examination due to its potential for toxicity. Several research has indicated a potential correlation between it and cancer, namely impacting the thyroid and other organs in experiments with rodents. Nevertheless, these discoveries have not been definitively reproduced in human subjects. The FDA and EFSA regulatory authorities have examined the data and determined that Acesulfame-K is safe for human consumption as long as it is within the recommended daily intake ADI guidelines. There are concerns that it may interfere with metabolic processes and change the composition of gut bacteria[21]. However, current studies do not definitively show major health consequences when consumed normally.

2.3 Cyclamate

Cyclamate, a compound with a bitter aftertaste, was first discovered in 1937 by Michael at the University of Illinois, USA. It originates from N-cyclo-hexyl-sulfamic acid (CHS). It has a sweetness level of 30 times more than sucrose and is utilized in the beverage and food sector as an artificial sweetener[22]. It is both water-soluble and alcohol-soluble and more stable than other sweeteners. In 1970, the United States prohibited it because of its linked health issues. Liver biopsies from diabetic individuals with cyclamate consumption showed no signs of triglyceride buildup or glycogen deposition. However, chronic investigations revealed a slightly higher occurrence of nephritis and nephrosis, which were also frequent in control rats. Cyclamate mostly causes kidney calcification and hyperplasia. Nephrocalcinosis was detected in 5% of the control group and 47-49% of rats administered cyclamate[23]. The most reported effects in animals and humans include softening stools and diarrhea. Ingestion of cyclamate at 5g per day dosages often resulted in loose, bulky stools. Animals also exhibited localized calcified lesions in the heart muscle, accompanied by degeneration and necrosis.

2.4 Neotame

Neotame is the latest artificial sweetener derived from aspartame. The FDA approved it in 2002, as confirmed by the US in 2002[24]. According to the European Food Safety Authority (2007), it has no calories and is 6000-10,000 times sweeter than sucrose and 30-60 times sweeter than aspartame. The invention of this compound may be attributed to French chemists Nofre and Tinti, who derived it from the combination of dipeptide phenylalanine and aspartic acid. The chemical structure of this substance closely resembles that of aspartame. It is commonly used as a sweetener in soft drinks, jellies, processed fruits, syrups, chewing gum, and gelatins[25]. It is also used in cooking and baking since it can withstand high temperatures. Nevertheless, a small quantity is assimilated and processed. The body weight associated with consuming this sweetener is attributed to its toxicity and poor palatability, leading to decreased food intake.

2.5 Saccharin

Saccharin is approximately 300 times more sweet than sucrose, according to studies conducted by Bizzari[26]. The substance undergoes decomposition at a temperature of 228°C when exposed to acid, as reported by Lide in 1997. When subjected to sodium and calcium salts, the substance disintegrates at temperatures above 300°C. The accidental discovery of saccharin, the first artificial sweetener, was made by Remsen and Fahlberg in 1878. Saccharin's inexpensive manufacturing cost and unavailability of pure cane sugar led to its widespread use until World War I Arnold researched two successive saccharin bioassays. The findings indicate that the generation of humeral antibodies in rats is significantly impaired, possibly contributing to cancer development. In

1977, the FDA suggested prohibiting the use of saccharin because of reports of cancer in experimental rats[27]. 2000, the prohibition was lifted according to the Calorie Control Council (2007). However, it remains in effect in Canada, as Health Canada (2007) stated. The study by Negro et al. (1994) found that exposure to pure saccharin contributes to the development of liver damage. Multiple investigations have demonstrated a correlation between bladder cancer and saccharine, as evidenced by the research conducted by Fukushima et al. in 1986 and Cohen et al. in 1991. The consumed saccharin is eliminated from the body through urine after it circulates in the bloodstream.

2.6 Sucralose

Sucralose is a non-caloric sweetener that is 600 times sweeter than sucrose, according to the International Food Information Council (2005)[28]. The molecular weight of sucralose is 400.The taste of sucralose resembles that of cane sugar and leaves no lingering bitter after taste. In 1976, two researchers employed by Tate and Lyle, a British sugar refining firm, stumbled onto this molecule by chance. They were surprised to find that this newly discovered chemical had an unusually high level of sweetness. The trademark name of the product is Splenda, and it received approval from the FDA in 1999[29]. Studies conducted on animals have demonstrated that sucralose can lead to various issues, including enlarged liver and kidneys, reduced size of thymus glands (up to 40% decrease), increased weight of feces, shrinking of lymph follicles in the spleen and thymus, decreased red blood cells, slower growth rate, prolonged pregnancy duration, hyperplasia of the pelvis, terminated pregnancies, and diarrhea. In a study conducted by Bigal and Krymchantowski in 2006[30], it was shown that sucralose was a causative factor for migraines. The presence of the 6-chloro 6-deoxyglucose component in the chemical structure of the sweetener is the cause of the anti-fertility effect seen in rats. According to Japanese researchers, it has been asserted that consuming sucralose might cause harm to the DNA in the organs of the gastrointestinal system. Various studies have consistently demonstrated a significant link between the use of sweeteners and harmful effects on the liver, kidneys, fetal development, and the slowing down of placental growth.

2.7 Aspartame

Aspartame, was first identified in 1965 by James Schlatter, a chemist. The substance has L-aspartyl-L phenylalanine methyl ester, an artificial sweetener that does not include saccharide[31]. It is a methyl ester derived from the combination of the amino acids aspartic acid and phenylalanine. Aspartame can produce methanol through hydrolysis under very acidic or highly alkaline circumstances[32]. Under more rigorous circumstances, the peptide bonds are broken down by hydrolysis, forming individual amino acids. At room temperature, it has a little solubility in water, approximately 3 grams per 100 milliliters, with a pH of 3. The solubility is directly influenced by pH and temperature. It rises with both higher and lower pH levels, as well as with increased temperature. The stability of aspartame in an aqueous solution follows a bell-shaped curve concerning pH, with the highest stability seen at pH 4.3[33]. This sweetener is commercially promoted under several brands, such as Equal, Nutrasweet, and Candere. It possesses a pleasant and pure sweet flavor, although its duration and level of sweetness vary compared to sucrose. The main issue regarding aspartame's neurotoxicity is mostly due to its conversion into the necessary amino acid phenylalanine during metabolism[34]. Phenylalanine is a necessary amino acid that humans must consume daily, with a recommended intake of around 12 mg per kilogram of body weight. The average daily phenylalanine intake in adults' regular diet in the United States is around 50 mg/kg. Elevated levels of phenylalanine in the blood plasma are responsible for around 1% of cases of intellectual disability in randomly chosen groups in the United States. The condition arises from a genetic mutation in the enzyme phenylalanine hydroxylase, which catalyzes the conversion of phenylalanine to tyrosine. Phenylketonuria (PKU) occurs when large levels of phenylalanine are discharged in the urine in the homozygous type[35]. Ingesting aspartame leads to a rise in the levels of the excitatory amino acids aspartate and phenylalanine in the bloodstream. In extremely high amounts, it may also raise the concentration of phenylalanine in the brain. Applying aspartate or glutamate directly to neurons can cause neuronal depolarization and cellular bursting in animal model systems[36]. However, it would be erroneous to assume that increasing the plasma concentration of aspartate or phenylalanine would cause convulsions in living beings. The breakdown of APM starts in the oral cavity when salivary enzymes initiate hydrolysis as a protein. Unlike the metabolites of sucrose, such as glucose, fructose, and lactic acid, the metabolites of APM, namely aspartic acid and phenylalanine, do not substantially impact the decay process as cariogenic agents[37]. The potential for ogenic activity is theoretically present. Therefore, it is reasonable to anticipate that the cariogenicity of APM would be very low, making it a potent innovation in preventive dentistry when employed as a substitute for sucrose. It is important to exercise caution before becoming too enthused about the possibilities. Due to APM's susceptibility to high heat and long-term exposure, it is improbable that APM will ever fully replace sucrose as a stabilizer. APM also has a lower sucrose content, which is advantageous for certain goods. Given this understanding, several research has been conducted to investigate the impact of APM in combination with glucose or sucrose[38]. Various artificial sweetners shown in figure 1.

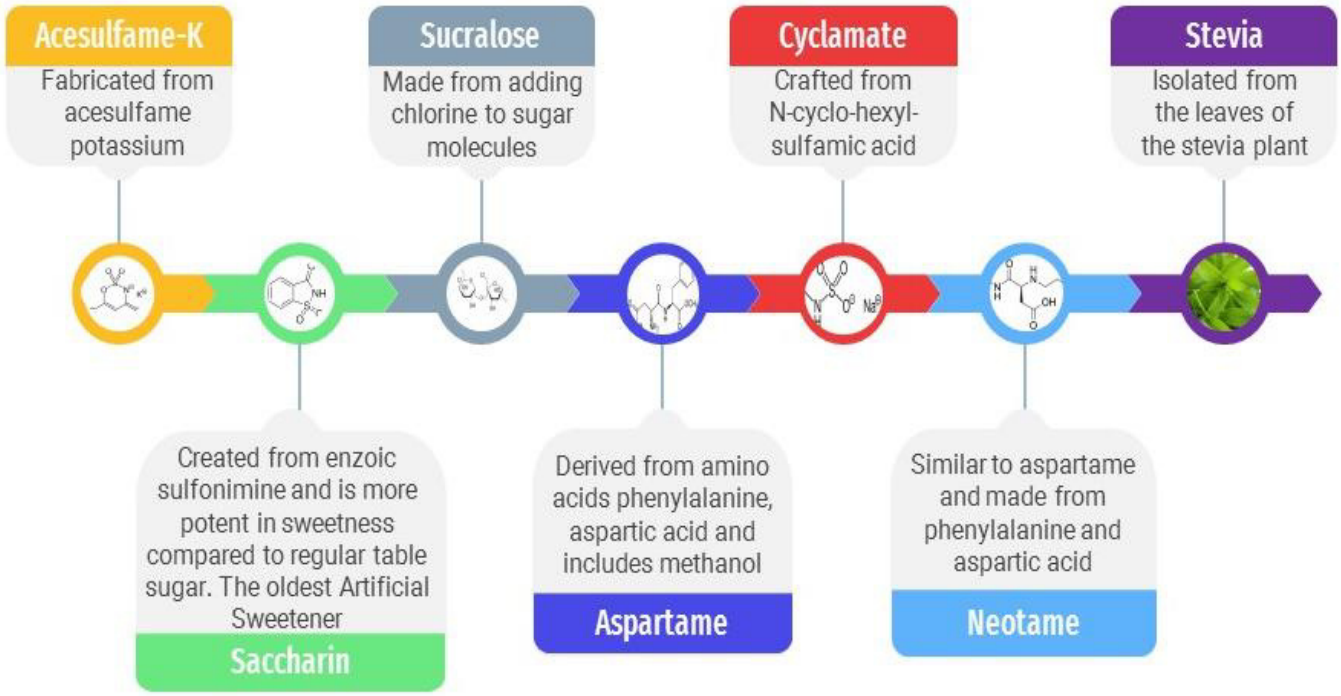

Fig 1: Various Artificial Sweetners

3 RISK OF CARDIOVASCULAR DISEASES, CANCER, AND IMMUNITY

Several scientific studies, including experiments conducted on living organisms (in vivo) and in laboratory settings (in vitro), as well as observational studies and controlled trials involving humans, have investigated the impact of consuming artificial sweeteners or artificially sweetened beverages on early cardiovascular and cancer risk indicators[39,40]. These indicators include weight status, hypertension, inflammation, vascular dysfunction, and disruption of gut microbiota. Most of these research indicated adverse effects, whereas just a small number demonstrated neutral or beneficial characteristics. This study's findings indicate a direct correlation between elevated risk of cardiovascular disease and greater consumption of artificial sweeteners, including aspartame, acesulfame potassium, and sucralose. Several variables, including race and ethnicity, genetic inheritance, tobacco use, and alcohol use, can influence colorectal cancer (CRC) [41]. There has been a longstanding hypothesis that food additives such as saccharin and sucralose, which hinder the growth of gut bacteria, may have played a significant role in causing Inflammatory Bowel Disease (IBD). These additives compromise the ability of deconjugated bilirubin to inactivate digestive proteases, which are normally facilitated by bacterial b-glucuronidase. The influence of sweeteners on several blood biochemical parameters, enzyme activities, and immunological parameters in male and female albino mice following 8 and 16 weeks of sweetener use were reported[42]. The sweeteners were given to male and female mice as a solution that had been sweetened for five hours daily. Both preferred consuming water that was sweetened with either Stevia or sucralose. Both sweeteners significantly reduced the levels of hemoglobin, HCT%, RBC, and WBC counts. After 18 weeks, both non-caloric sweeteners substantially impacted the liver and kidney function enzymes in both male and female mice[43]. The biochemical findings were confirmed by histological examination in the groups that received sucralose and stevia, revealing significant harm to the liver and kidney tissues. Administering sugar to male mice resulted in a modest rise in their ALT, AST, and cholesterol levels. Male and female mice that were administered sucralose or stevia saw a significant rise in the levels of several immunoglobulins (IgG et al.) and pro-inflammatory cytokines (IL-6 and -8), along with a notable drop in the level of the anti-inflammatory cytokine IL-10[44]. Conversely, sugar administration resulted in an elevation of IgA levels and a reduction in IL-10 levels.

4 NATURAL SWEETENER-A POSSIBLE OPTION

Natural sweeteners are chemicals derived from plants or natural sources that have a sweet taste and provide some nutritional benefits. The extraction procedure of natural sweeteners does not include any chemical modification. Natural sweeteners have gained widespread popularity, and their manufacturing and extraction methods have been refined and improved.

4.1 Stevia

Stevia (Stevia rebaudiana), a perennial plant in the Asteraceae family, is popularly referred to as "sweet leaf," "sweet herb," and "honey leaf." The initial discovery of this phenomenon was made by M.S. Bertoni in the year 1887[45]. The leaves of the stevia plant contain around 10 glycosides that have sweetening properties. The most significant ones are Stevioside (3-10%), Rebaudioside A (13%), and Rebaudioside B, C, and D. China, Thailand, Paraguay, Taiwan, Malaysia, Korea, Japan, and Brazil are the primary nations that produce stevia. According to Thomas and Glade (2010), Stevia is significantly sweeter than cane sugar, with a sweetness level that is 250-300 times higher. Stevia has zero calories and is considered to be very safe for use without undergoing any processing. Stevia is a natural sweetener derived from the stevia plant and does not suffer any chemical alteration during production[46]. It caters to

numerous customers seeking healthier alternatives to sucrose sugar. Presently, Stevia is utilized in Brazil, Korea, Israel, the United States of America, Japan, China, Canada, and Paraguay in the bakery, confectionery, and beverage industries, as well as in domestic items. Several researchers advocate it. Research has indicated that stevia is safe for those with phenylketonuria and diabetes compared to other sweeteners. In addition to being a non-calorie sweetener, steviol glycosides have taste-enhancing qualities that contribute to their appeal in drinks and food products. Stevioside and other compounds, such as rebaudioside A and glucoside, provide sweetness and have therapeutic effects. These effects include reducing inflammation, lowering blood sugar levels, reducing high blood pressure, fighting against cancer, preventing diarrhea, modulating the immune system, and promoting urine production (Chatsudthipong & Muanprasat, 2009) [47]. Steviol can modulate drugs by interacting with drug transporters. In the 1990s, Stevia was prohibited in the United States unless labeled as a "supplement." In 2008, two businesses filed FDA applications to obtain GRAS (Generally Recognized as Safe) status for a 95% pure Rebaudioside A (Reb A) extract. The Food and Drug Administration (FDA) issued a "no objection" notice to both firms (FDA, 2008), and the initial products containing the rebaudiana extract were introduced to the market that same year (Calorie Control Center, 2008). In September 2009, the French country became the initial European Union (EU) to approve using Stevia extracts containing a minimum of 97% Rebaudioside A (Reb A) as sweeteners in food and beverages[48]. Stevioside and stevia extracts have received formal approval as food additives in Korea, Brazil, and Japan. The FDA affirms the safety of rebaudiana for human consumption based on rigorous research that has undergone peer review and comprehensive safety investigations conducted over many generations. In 2006, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) declared a provisional acceptable daily intake (ADI) of stevioside of up to 5.0mg/kg of body weight (JECFA, 2006). Pepsi and Coca-Cola are the two firms that obtained patents for stevia products, which they market under PureVia and TruVia[49]. Summary of this review shown in figure 2.

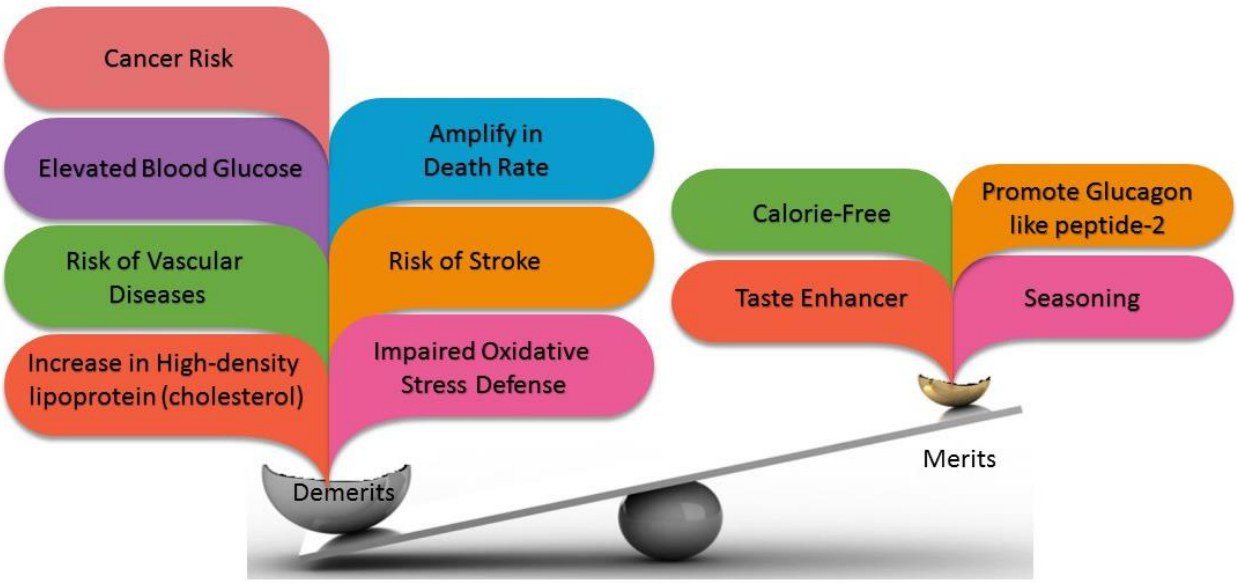

Fig 02: Merits and De Merits of Artificial sweeteners

5 CONCLUSION

Recent extensive research on the general and reproductive toxicity of stevioside has shown that it is safe even when consumed at high doses in the diet. Furthermore, there is no evidence or presence of genotoxicity and allergic responses associated with stevioside, as stated by Qing Yang in 2010. In the future, Stevia rebaudiana can be used as an additional component in oral care products such as mouthwash, toothpaste, chewing gum, artificial saliva, and chewable tablets. Considering its medicinal advantages, it is a boon and particularly advantageous for those who are obese, diabetic, or have hypertension. The research given in this review paper indicates that using artificial sweeteners is linked to many negative health consequences. Regular intake of NAS (non-caloric artificial sweeteners) is associated with exacerbating glucose intolerance, which raises concerns over their effects on metabolic health. Moreover, the possible hazard of cardiovascular ailments linked to artificial sweeteners emphasizes the necessity for additional investigation in this field to determine causation. The analysis also underscores the hazardous capacity of several frequently employed artificial sweeteners, underscoring the significance of cautious deliberation while using these drugs. The correlation between artificial sweeteners and colon cancer elicits apprehension about their enduring safety. Furthermore, the adverse impacts of artificial sweeteners on the immune system and the potential correlation with bladder cancer underscore the significance of closely monitoring and controlling their inclusion in food items. This review paper emphasizes the importance of being cautious and conducting further research on the safety of artificial sweeteners despite their promotion as sugar alternatives for those seeking to reduce calorie consumption or control diabetes.

6 AUTHOR CONTRIBUTION STATEMENT

Dr. John Abraham contributed to the conceptualization and methodology of the study and was responsible for the original draft preparation. Dr. Gurshan Singh Gill handled data curation and formal analysis and played a key role in reviewing and editing the manuscript. Dr. Asit Kumar supervised the project, managed the administration, and secured funding for the research. Y. C. Tripathi conducted the investigation, provided resources, and worked on the visualization of the data.

7 CONFLICT OF INTEREST

Conflict of interest declared none.

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