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The development and characterization of a plant-based functional beverage using Rebaudioside-A

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ABSTRACT

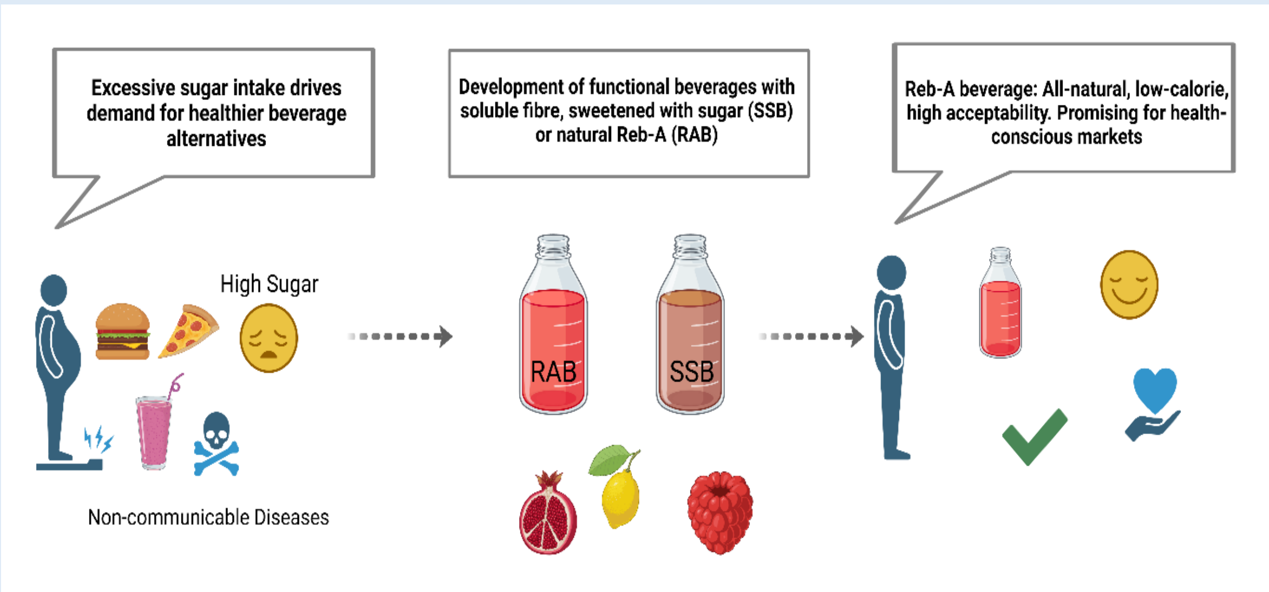
Background: Excessive sugar intake is a major global health issue, fuelling non-communicable diseases and driving consumer demand for nutrient-rich, low-calorie beverages without artificial additives. Functional beverages (FB) present an effective solution by conveniently delivering targeted health benefits. Rebaudioside-A (Reb-A), a natural, zero-calorie sweetener derived from *Stevia rebaudiana*, provides a promising alternative to sugar and artificial sweeteners, enabling the development of all-natural, low-calorie FBs.

Objective: This study aimed to develop a FB with soluble fibre, in two versions: one sweetened with sugar (SSB) and another with Reb-A (RAB), and compare their sensory, physicochemical, and functional properties.

Methods: Both SSB and RAB were prepared from pomegranate, raspberry, and lemon juices, as well as green tea, coconut water, and inulin. The beverages were pasteurized (85°C, 1 min) and stored at 3–4°C. Analyses included pH, total soluble solids (TSS), water activity, colour, antioxidant properties (FRAP, total phenolic content - TPC, DPPH, and total anthocyanin content - TAC), and sensory evaluation using a 9-point hedonic scale for taste, odour, colour, and overall acceptance.

Results: The RAB finished product demonstrated higher antioxidant activity, with higher DPPH scavenging ($p<0.05$) and increased FRAP values ($p<0.05$) compared to SSB. Both beverages presented the same pH, TPC, TAC, water activity levels, and inulin content (5.5 g/serving). Sensory evaluations revealed an overall preference of 70.5% for SSB ($p = 0.001$), although female participants (42% of the cohort) showed no significant preference. Acceptability indices were high for both (SSB: 83.4%, RAB: 76.9%; $p = 0.003$), with RAB scoring above 6/9 across all attributes. There were no differences in colour or odour between the two beverages

Novelty of the Study: This study is among the first to systematically develop and compare functional beverages (FBs)



Graphical Abstract: The Development and Characterization of a Plant-based Functional Beverage using Rebaudioside-A. Created with BioRender.com

INTRODUCTION

The consumption of sugar is increasingly widespread across the population, largely due to its presence in a wide variety of foods and beverages [1]. Excess sugar consumption has been related to the development of many chronic diseases such as obesity, Alzheimer's, type 2 diabetes mellitus (T2DM), and cardiovascular diseases (CVDs) [2-5]. To combat the rising challenge of excessive sugar consumption, both governments and manufacturers have introduced a range of preventive strategies, such as sugar taxes and reliance on sugar substitutes [6,7]. Sugar substitutes—including artificial sweeteners such as aspartame and sucralose, as well as plant-based options such as stevia and monk fruit—offer the sweetness of sugar with fewer or no calories [8]. These strategies, when combined, aim to lower sugar consumption at both the population and individual levels, promoting better public health outcomes.

Sugar-sweetened beverages (SSB) are the largest source of added sugars in the diet with a 355 mL serving of soda typically providing 150 calories and 35g of sugar [9] Consumers' awareness of sugar's involvement in chronic diseases and weight gain has driven significant expansion in the low- and no-calorie beverage market, promoting the use of sweeteners in the development of beverages [10]. Artificial sweeteners have been previously reported to cause adverse health implications such as gastrointestinal symptoms, cardiovascular impacts, neurological effects, and cancer [11-14]. Consumers are increasingly seeking drinks that not only eliminate added sugars but also incorporate natural sweeteners, offering a healthier alternative without compromising on taste [15-17] The trend of all beverages for the future is that they are sugar-free or naturally-sweetened, while enhancing health [18-19].

Stevia *(Stevia rebaudiana* Bertoni) is an ancient, perennial shrub of the Asteraceae family, commonly found in South America, and has been used for generations as the "sweet leaf", as its leaves synthesize sweet compounds known as steviol glycosides [20]. Rebaudioside-A (Reb-A) is a steviol glycoside extracted from the leaves of the stevia plant which is 250–450 times sweeter than sugar, and is a useful natural sweetener for beverage production [20-21]. Approved in the EU as food additive E960, Reb-A is considered safe, with an acceptable daily intake (ADI) of 4 mg/kg body weight [22-23]. Besides sweetness, Reb-A has previously displayed antioxidant properties and has exhibited beneficial effects towards obesity, diabetes, and cardiometabolic health when consumed in safe doses [24-27]. It provides sweetness without adding calories, supporting the development of functional foods [21]. Reb-A is more potent at lower temperatures, enhancing its suitability for beverages [21]. Although some steviol glycosides can have bitter or licorice-like aftertastes, they are widely used in beverage development, with Reb-A being preferred for its cleaner taste, to reduce sugar in drinks, thereby contributing to better public health [28-30].

A functional beverage (FB) is any non-alcoholic drink that provides health benefits beyond basic nutrition via bioactive components from plant, animal, marine, or microbial sources [31]. The global FB market is projected to reach over $208 billion by 2027 [32]. These beverages aim to support immune, gut, and cardiovascular health, or assist with weight management and type-2 diabetes [18,33-37]

Plant-based FBs made from fruit and vegetable juices are rich in essential nutrients and phenolic compounds that can exert positive health benefits [31]. Some of the more prominently used ingredients in these FBs are 'superfruits' as they are rich in bioactive compounds that have antioxidant effects [34]. Plant-based beverage production commonly involves combining fruits, vegetables, and herbs with other ingredients, such as fibre, minerals, and vitamins [34]. Thus, a variety of fruit juices, green tea, and a prebiotic fibre were selected for this FB development, as their combination is beneficial for developing a nutrient-dense functional beverage (FB) [38]. Pomegranate *(Punica granatum L.)* juice is known to have a high polyphenolic content (anthocyanins, gallic acid, flavonoids, ellagic acid and others), which are linked to antioxidant, anti-inflammatory, and cardioprotective effects [39-42]. Raspberries (*Rubus idaeus L.*), known for their high vitamin C content (28–34 mg/100 g) and consumer-friendly flavour, help achieve the recommended daily vitamin C intake for Irish adults (40 mg) [43-45]. Lemon (*Citrus limon L*.) juice contains vitamin C, citric acid, and polyphenols, including eriocitrin, which offers intestinal benefits and acts as a natural preservative that limits enzymatic browning in pomegranate juice [46-48]. Lime juice can mask the bitter aftertaste of stevia, thereby improving the taste; therefore, lemon juice was used to attempt to replicate this effect [49]. Coconut (*Cocos nucifera L*.) water provides electrolytes and supports hydration and digestion [50-52]. Green tea *(Camelia sinensis)* supplies caffeine and catechins, especially epigallocatechin-3-gallate (EGCG), and has demonstrated benefits for cancer, hypertension, obesity, diabetes, and Alzheimer's disease when consumed in recommended amounts [53-62].

Dietary fibre is a vital component of a healthy diet, and its inclusion in a beverage would prove beneficial for public health [63-65]. Development of a FB to target the common fibre deficiency in Irish adults, who average 19 g/day compared to the recommended 25 g/day could help reduce the risk of colorectal cancer [64,66,67]. Inulin, a prebiotic fibre, supports beneficial gut bacteria and improves metabolic health, satiety, and mineral absorption, and is a perfect candidate to add to beverages to provide health benefits [68-69].

This study aimed to develop a novel Reb-A sweetened beverage (RAB) for the Irish market and compare it to a traditional SSB of the same ingredients to assess its market potential. These all-natural FBs were compared and evaluated in terms of their sensory, antioxidant, and physicochemical properties to characterise the beverage's potential health effects and properties.

MATERIAL AND METHODS

Ingredients: Reb-A sample (98% pure) was purchased from the KoRo company(Berlin, Germany). Inulin was provided by Orafti HP® Inulin (Beneo, Belgium). Green tea (Barry's, Cork, Ireland), coconut water, caster sugar, raspberries (Driscolls Maravilla variety), lemons, and pomegranates were purchased from local supermarkets.

Chemical Reagents: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), gallic acid, and ascorbic acid were obtained from Sigma-Aldrich-Merck (Darmstadt, Germany). Hydrochloric acid (HCl) was purchased from Fluka (USA). Folin-Ciocalteu's phenol reagent, methanol, and ferric chloride hexahydrate were acquired from Merck KGaA (Darmstadt, Germany). Potassium chloride, acetic acid (glacial), sodium carbonate, and sodium acetate were purchased from Scientific Laboratory Supplies (Dublin, Ireland). Ultra-pure water was used in the analytical determinations.

Beverage Preparation: Two beverages were prepared: a RAB and a traditional SSB (control). Fruits were juiced (lemon and pomegranate by hand-press, raspberries by blender), filtered (Mesh size 1.5 mm), and measured into relevant portions. Green tea was brewed (6 bags in 100 mL water at 60 °C for 3 minutes) and combined with the juices and coconut water. Inulin was added with stirring, followed by sugar or Reb-A as appropriate. Pre-sterilized bottles were filled, pasteurized at 85 °C for 1 minute in the 'Milky FJ 15 (230V) pasteurizer, rapidly cooled, and stored at 3–4 °C.

The ingredient composition of RAB and SSB was systematically adjusted to ensure both formulations yielded an equivalent final volume of 250 mL. While both beverages share core components—coconut water, pomegranate juice, lemon juice, raspberry juice, green tea, and inulin—their relative proportions differ. RAB contains higher volumes of each juice and coconut water compared to SSB, reflecting increases in their respective percentages (e.g., coconut water: 58.7% in RAB vs. 51.05% in SSB). Notably, SSB utilizes sugar (13.18%) as a sweetener, whereas RAB excludes sugar and instead incorporates Reb-A (0.07%), a stevia-derived sweetener. These modifications in ingredient quantities and sweetener types were made to compensate for the absence of sugar in RAB, thereby maintaining the same total beverage volume in both formulations (Supplementary Table 1).

Physicochemical analysis: *pH, Total soluble solids (TSS), and Water activity (a_w)*

The pH analysis was conducted using an Eti Ltd 8000 pH meter (Scientific Laboratory Supplies, Dublin, Ireland), and the brix degree (°Brix) was used as an indicator of TSS percentage with a portable VWR ATC handheld refractometer (VWR Avantor, Dublin, Ireland). The a_w of the beverages was measured using an AquaLab Series 3 water activity meter (Decagon Devices Inc, Pullman, Washington, USA). *Inulin Stability and Sugar Analysis*

The stability of inulin was evaluated through sugar composition analysis. Sugar analysis was performed by Beneo GmbH (Mannheim, Germany) using High-Performance Liquid Chromatography (HPLC), following the official AOAC method 997.08. This method is specifically validated for the quantification and characterization of inulin and related sugars.

Colour analysis: Colour of the beverages was measured by reflectance using a portable colourimeter (Model CM-700d, Konica Minolta), following the protocol by [70], and calibrated using a standard white reflector plate where white L* = 100. Results were expressed using a CIE (L*, a*, b*) system where L* - lightness, a* (redness-greenness), b* (yellowness-blueness) The colour intensity (C*) and the total colour difference (ΔE*) were calculated using the following formulas from [71,72].

- Colour Intensity (C*):

$$\boxed{(C^*) = \sqrt{[(a*)^2+(b*)^2]}}$$

- The total colour differences (ΔE*):

$$\boxed{(\Delta E^*) = \sqrt{[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]}}$$

[Where $\Delta L^* = (L2^*-L1^*)$, $\Delta a^* = (a2^*- a1^*)$, and $\Delta b^*=(b2^*-b1^*)$]

Antioxidant analysis: Antioxidant analysis of the final beverages formulated (SSB and RAB) was carried out. Estimated values were calculated for the normalized RAB formulation (Supplementary Table).

FRAP determination: The ferric reducing antioxidant power (FRAP) determination was performed following the method described by [73]. Freshly prepared FRAP reagent and samples were incubated in a 96-well plate in the dark for 30 minutes. Absorbance was measured at 593 nm, and results were expressed as ascorbic acid equivalents (mg AAE/L).

Total phenolic content (TPC): The samples were assessed for their TPC using the Folin-Ciocalteau (FC) assay, following [74,75]. Samples (25 µL) were added to 200 µL of pure water and 25 µL of the FC reagent. After 5 minutes, a 10% sodium carbonate solution was added (25 µL). The absorbance was measured at 725 nm after a 60-minute incubation in the dark. The results were expressed as gallic acid equivalents (mg GAE/L).

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay: This analysis was conducted following [76] with some modifications. Samples (40 µL) was mixed with 260 µL of a 0.10 mmol/L methanolic solution of DPPH, and incubated in the dark for 30 minutes at 25 °C. The absorbance was measured, and results were expressed as ascorbic acid equivalents (mg AAE/L).

Total monomeric anthocyanins content (TAC): TAC determination was conducted using the differential pH method as described by [77] with modifications. The sample (20 µL) was mixed with 180 µL of a pH 1.0 (0.025 M) solution of potassium chloride and 180 µL of a pH 4.5 (0.04 M) solution of sodium acetate separately. The absorbance was measured at 520 and 700nm spectrophotometrically, results were expressed as mg of cyanidin-3-glucoside equivalents per L of beverage (mg CYE/L)

Sensory analysis: Ethical approval was granted for this study under the number [2024_06_07_S&E] by the Faculty of Science & Engineering Research Ethics Committee at the University of Limerick.

A sensory analysis study was conducted to assess the acceptance of the beverages and to analyse the preference between the traditional SSB and the RAB. Panellists completed an acceptance test using a 9-point hedonic scale, ranking the samples from 1 (dislike extremely) to 9 (like extremely) to rate the colour, taste, odour, and overall impression. The panellists also participated in a preference test, indicating which sample they preferred. The sensory trials were conducted in sensory booths and all samples were coded with different three-digit numbers to ensure blinding. Each panellist was provided with two shot glasses containing 15 mL of the different beverages (SSB, RAB).

The acceptability index (AI) was calculated to assess the overall acceptability of each parameter for each beverage using the following formula:

$$\mathbf{AI\ (\%) = A \times 100/B}$$

(A = mean grade obtained for the product; B = maximum grade given to the product)

Statistical analysis: Data was tested for normal distribution using the Shapiro-Wilks test, to distinguish data between parametric and non-parametric, and appropriate tests were performed. The results obtained throughout the experiment were subjected to analysis of variance (ANOVA) for parametric data, or the Mann-Whitney U for non-parametric data to determine whether there was a statistical significance between samples ($p<0.05$) using SPSS statistical software version 29.0.1.0 (171).

RESULTS

Physicochemical Analysis: Table 1 presents the results of the pH, TSS (°Brix), and water activity of the RAB and SSB after pasteurisation. The data before Pasteurisation can be seen in Supplementary Table 2.

Table 1. pH, water activity, °Brix and Inulin stability values of SSB and RABs after pasteurisation.

Test	Beverage type	After Pasteurisation
pH	SSB	3.39 ± 0.00
	RAB	3.39 ±0.00
Water Activity (a_w)	SSB	0.97 ± 0.00
	RAB	0.98 ± 0.01
°Brix	SSB	20.50 ± 0.71
	RAB	8.05 ± 0.07
***Sugar content* (g/100 mL of sample)**		
Fructose	SSB	4.0
	RAB	2.0
Glucose	SSB	3.9
	RAB	1.8
Sucrose	SSB	8.7
	RAB	0.0
***Inulin stability* (g/100 mL of sample)**		
Fructan	SSB	2.2
	RAB	2.2

Note: Results for pH, Water Activity (aw), °Brix are expressed as the mean ± standard deviation (SD) as results were measured in triplicate. *ANOVA was applied to assess differences between beverages and the effect of pasteurisation as data is parametric).*

After pasteurization, both the SSB and RAB exhibited identical pH values of 3.39. The water activity was significantly higher in the Reb-A beverage (0.98 ± 0.01) compared to the Sugar beverage (0.97 ± 0.00) (p-value<0.001). Similarly, the °Brix value was significantly higher in the SSB (20.50 ± 0.71) than in the RAB (8.05 ± 0.07). Overall, while the pH remained consistent between the two beverages, the RAB showed significantly increased water activity and lower °Brix compared to the SSB.

Inulin stability results indicate that both the SSB and RABs contained 2.2 g of inulin per 100 mL, which equates to 5.5 g per 250 mL serving after pasteurization. Sugar analysis revealed that the SSB contained a substantially higher sugar content, reflecting the added sugars in its formulation. The RAB, on the other hand, contained only a small amount of naturally occurring sugars, consistent with its use as a non-nutritive sweetener.

Colour analysis: Colour analysis is important in beverage development as it affects the judgement of consumers acceptance of a product, quality perception, and purchase decisions [78,79] . The beverage was pink-coloured which was attributed to the anthocyanins from the pomegranate and raspberries [80-82].

Table 2. Colour values (L*, a*, b*), colour intensity (C*), and total colour difference (ΔE*)

Beverage	L*	a*	b*	C*	ΔE*
SSB	24.88 ± 0.66	12.11 ± 0.82	3.81 ± 0.41	12.70	-
RAB	22.74 ± 1.30	11.73 ± 1.03	3.49 ± 0.51	12.24	-
SSB vs RAB	-	-	-	-	2.20

Note: Results for L*, a*, and b* are expressed as mean ± SD from triplicate measurements, Colour differences are unrecognizable (0 < ΔE* < 1), experienced observer can perceive the differences (1 < ΔE* < 2), inexperienced observer can perceive the differences (2< ΔE*< 3.5), Every observer can easily see the difference (3.5 < ΔE* < 5), and an observer recognizes two different colours (ΔE* > 5) [71].

After pasteurisation, the colour difference between both beverages was slightly noticeable for observers (ΔE*=2.20) and this difference had significantly reduced compared to the beverages before pasteurisation (See supplementary Table 3). An independent t-test revealed no significant differences in colour parameters (L*, a*, b*) between the two beverages (p-value > 0.05). Pasteurisation seemed to reduce the colour difference between the SSB and RAB, potentially due to the potential caramelisation of sugar in the SSB causing darkening [71].

Antioxidant analysis: The antioxidant potential of the SSB and the RAB were assessed through four different antioxidant assays. The summary of the results for each antioxidant assay is featured in Figure 1 and related data in Supplementary Table S4.

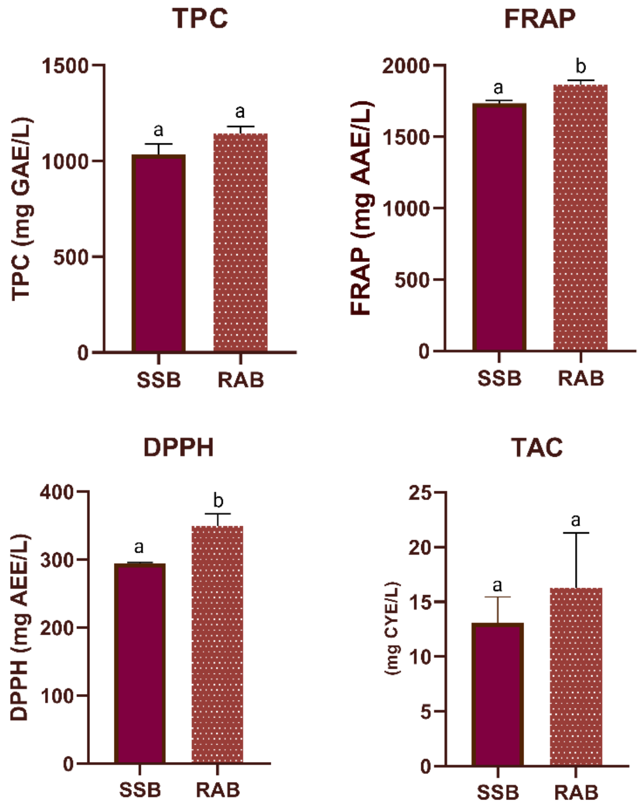


Figure 1. Antioxidant assay results for SSB and RAB. RAB exhibited significantly better DPPH radical scavenging and FRAP activities than the SSB (p-value < 0.05). Although the RAB has higher TAC values (16.31 ± 5.0 mg M3G/L) compared to the sugar beverage (13.08 ± 2.37 mg M3G/L), there is no significant difference in the TAC between the beverages (p-value > 0.05). (Created in BioRender. Hoffmann Sarda, F. (2025) https://BioRender.com/cqvl9s9) (Note: Statistical significance is determined by the letters a and b)

When comparing the beverages, there was no significant difference between the TPC of either beverage (p-value>0.05). However, the RAB exhibited significantly better DPPH radical scavenging and FRAP activities than the SSB (p-value < 0.05). Although the RAB has higher TAC values (16.31 ± 5.0 mg M3G/L) compared to the sugar beverage (13.08 ± 2.37 mg M3G/L), there is no significant difference between the beverages' TAC (p-value>0.05).

When antioxidant activities were also calculated on an equivalent fruit/tea extract basis (Supplementary Table S5), values for SSB and RAB were highly similar across all assays; however, given the higher fruit extract content in the RAB formulation, a typical serving would deliver greater total antioxidant activity in a real-life consumption scenario.

Sensory analysis: A sensory analysis was conducted to compare the SSB and RAB with 103 participants aged 18 and above. Of the participants who conducted the trial, 15 participants' results were excluded from the analysis (incomplete answers), and 88 were included in the final analysis (See Supplementary Figure S1 for participant information). The average age of the 79 participants who entered results was 31.9 ± 10.6 as 9 participants did not disclose their age.

Sensory Acceptance Test: The results for the overall impression parameter were based on 84 participants as 4 people did not input any result. Summary of the sensory analysis results for each parameter for the SSB and RAB is provided in Supplementary table S6.

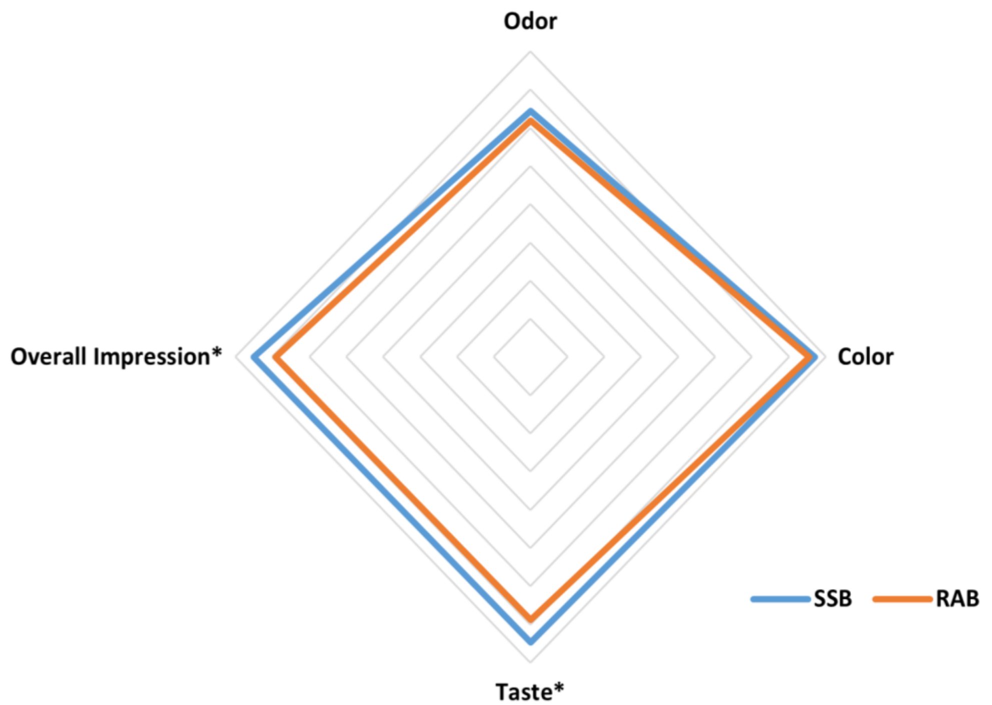


Figure 2. Spider diagram for the SSB and the RAB. This diagram displays the mean results for the following parameters: odour, colour, taste, and overall impression. Odour: No significant difference between beverages (p-value=0.310). Colour no significant difference in colour scores between the beverages (p = 0.591). There was a significant difference between the SSB and RAB's taste and overall impression scores (p=0.001), with the SSB receiving better scores.

Odour was the lowest-ranked parameter for both beverages, with no significant difference between beverages (p-value=0.310), but it showed the highest SD and CV values, indicating variability amongst participants' results.

There was no significant difference in colour scores between the beverages ($p = 0.591$), aligning with the minor colour difference observed ($\Delta E^* = 2.20$) in the colour analysis. Both beverages showed consistent and positive colour scores, with the lowest standard deviation, variance, and coefficient of variation.

There was a significant difference between the SSB and RAB's taste and overall impression scores (p=0.001), with the SSB receiving better scores. While some participants rated both beverages as excellent (score of 9), both also received low scores (minimum of 2) for taste, indicating high variability and diverse sensory perceptions among participants.

The mean scores for each parameter were higher overall for the SSB but the RAB still had positive sensory results. The spider diagram in Figure 2 illustrates the mean sensory analysis results for the SSB and RAB across each of the assessed parameters.

Effect of gender on acceptance test results: The sensory results were analysed to assess whether gender influenced the results. The analysis revealed no significant differences between male and female scores for odour, taste, or overall impression; however, a significant difference was noted concerning colour (p-value =0.047).

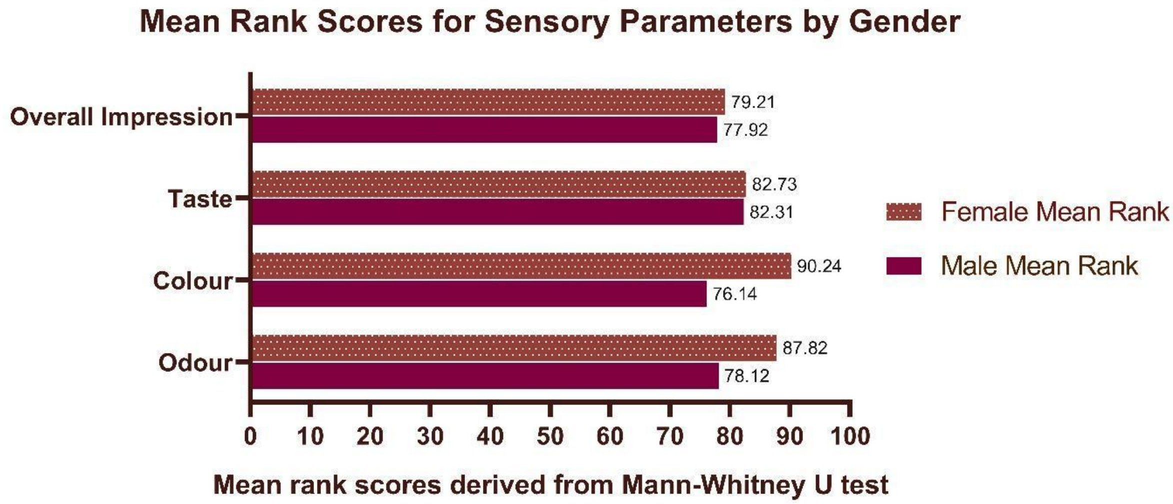


Figure 3. The mean rank scores for each sensory parameter for males and females derived from the Mann-Whitney U Test. A significant difference was observed in the colour ratings, with females rating the colour higher than the males (p = 0.047). No significant differences were found for the rankings of odour (p = 0.185), taste (p = 0.954), or overall impression (p = 0.853).

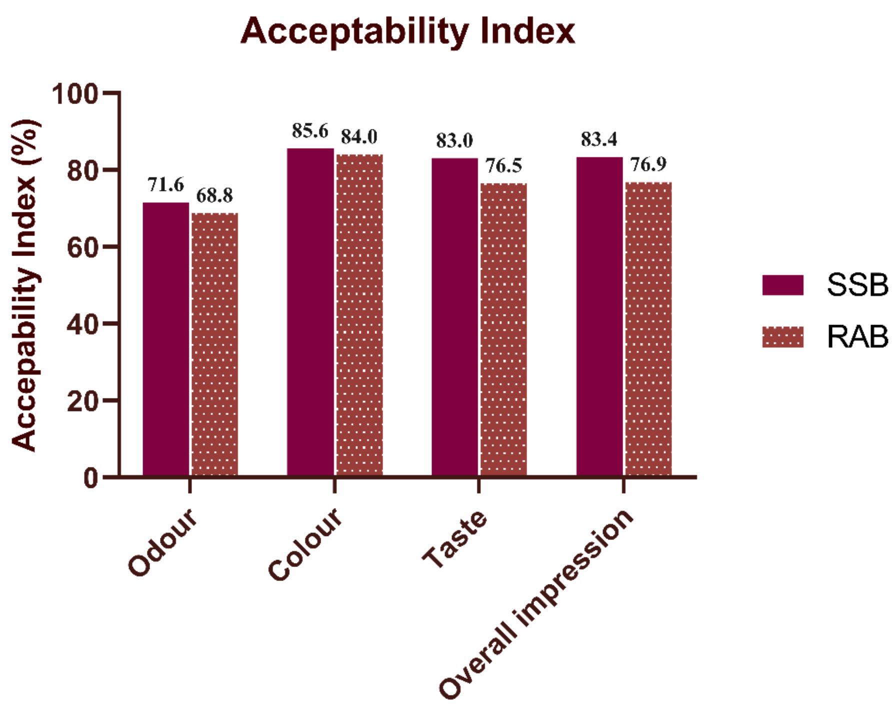


Figure 4. The AI (%) for odour, colour, taste, and the overall impression of both the SSB and RAB amongst the 88 participants of the sensory analysis. There were no significant differences between beverages odour (p-value=0.310). Significant differences were recorded in taste (p-value= 0.001) and overall impression (p-value= 0.003).

Acceptability Index

The acceptability index (AI) was calculated to assess the overall acceptability of each parameter for each beverage.

For a product to be considered accepted, the AI value should be >70% [83]. The SSB had higher AI values across all parameters compared to the RAB, displaying higher acceptability. The SSB's odour AI was 71.6%, while the RAB odour was 68.8%, which is near the acceptability threshold. As there were no significant differences between the beverages' odour (p-value=0.310), both are acceptable. Significant differences were recorded in taste (p-value= 0.001) and overall impression (p-value= 0.003).

Preference Test : 88 panellists completed a simple preference test by selecting their preferred sample, a method known to predict consumer behaviour without using numerical scales [84]. The minimum number of correct judgments needed for statistical significance, as outlined by Lawless and Heymann (2010) [85], is provided in Supplementary Figure S2.

62of the 88 participants preferred the SSB, accounting for 70.5% of all participants. The probability test confirmed that the SSB was significantly preferred over the RAB, as more than 57% of the participants preferred the SSB, which coincided with the AI result indicating that the SSB was more accepted. Of these 62 participants who preferred the SSB, 36 were male which is 80% of all males involved in the study. The SSB was preferred by 24 females (64.9%) but these numbers did not meet the threshold for statistical significance therefore, preference cannot be established. Research suggests females are generally more inclined to choose healthier options [86]. Overall, 26 out of 88 participants (29.5%) preferred the RAB, including 13 females (35.1%), 9 males (20%), and 4 undisclosed.

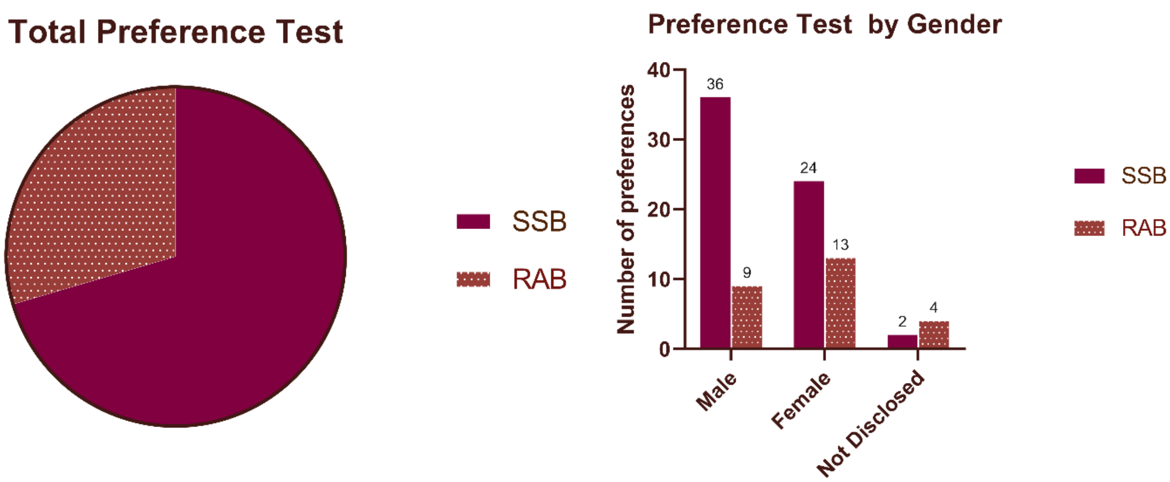


Figure 5. Preference of SSB and RAB; pie chart of summary (left) and bar chart summary of gender preference (right)

DISCUSSION

This study evaluated how sweetener type influenced the physicochemical, antioxidant, and sensory properties of two functional beverages—a sucrose-sweetened control (SSB) and a Rebaudioside A formulation (RAB)—and identified distinct differences between them. Both beverages maintained a low pH, ensuring microbial safety. The pH values for both beverages are below the critical pH value of 4.6, which is a recognised limit to control spore-forming pathogenic bacteria [87]. Raspberry juice is reported to have a pH value of 3.04, pomegranates have values ranging between 2.87 – 3.77, and lemon juice has low pH values (2.2-2.7) due to the high content of citric acid [88-91]. The inclusion of these fruits in the generation of a FB is advantageous for quality control as the FB is below the critical pH value.

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Water activity (a_w) is a crucial measurement in food quality control, as it is related to the stability and growth of microorganisms, as well as the chemical [92,93]. RAB consistently exhibited higher water activity than SSB, a result of sugar's well-known ability to lower water activity and thus inhibit microbial growth [94]. Although this could suggest a higher risk of microbial spoilage in RAB, the low pH of both beverages acts as a critical barrier, reducing this risk in practice.

Inulin stability is known to be affected by food processing factors such as pH and temperature, where at an acidic pH (1-3), hydrolysis of inulin increases with increasing temperature and heating time [95]. Several studies have displayed how the combination of thermal treatments and low-pH values of fruit juices leads to partial hydrolysis of inulin [96-98]. Therefore, given the low pH of both beverages (Table 1), it was essential to evaluate the stability of inulin after pasteurisation to ensure that the prebiotic fibre retained its potential functionality for this beverage to be classed as an FB. Given that even following pasteurisation, there was no change in the inulin content, this shows that inulin can be added to such FBs to increase the fibre content in the product.

In terms of antioxidant properties, RAB outperformed SSB, exhibiting a significantly greater DPPH radical scavenging capacity and higher FRAP activity. This enhanced antioxidant profile may be attributed to the higher percentage of fruit extracts added in the RAB, which helps compensate for the sugar volume in SSB. A comparative study comparing commercial FBs displayed that total polyphenols ranged from 27 and 398 mg GAE/100 mL, with only four beverages of 18 having higher quantities of TPC than the FBs developed in this study [31]. This demonstrates the suitability of these ingredients in creating a beverage rich in polyphenolic compounds which remain thermally stable.

Pasteurisation significantly improved the DPPH radical scavenging ability of both the SSB and RAB ($p<0.01$). Similarly, a previous study displayed that pasteurisation did not significantly increase TPC of pomegranate juice but had significant changes on the DPPH values [99]. This could be related to the inhibition of the polyphenol oxidase enzyme in juice, promoting the release of phenolic compounds from the plant cells into the juice which contribute to antioxidant activity [100]. The RAB had significantly better DPPH results than the SSB (p-value<0.05), and similar findings were observed in guava nectar sweetened with stevia compared to sugar [101].

There was a significant increase in FRAP values after pasteurisation in the RAB (p-value <0.05) but not in the SSB. Thus, the effect of thermal processing in releasing antioxidant compounds might be hindered by a higher quantity of sugar. The addition of sugar to green tea previously displayed significant decreases in its antioxidant activity, potentially due to the formation of glucose-gallic complexes [102,103]. However, the addition of stevia to tea displayed no significant effect on the antioxidant activity, accentuating its advantages as a sweetener [103]. Due to the high content of GT in this FB, the formation of these complexes could potentially explain why the RAB has significantly better DPPH scavenging and FRAP compared to the SSB.

Although the RAB has a trend of higher TAC values, there is no significant differences between the beverages (p-value>0.05). This finding contradicts the literature as several studies have reported significant losses in TAC following pasteurisation [104-106]. Anthocyanins colour changes to yellow or colourless due to temperature susceptibility [107,108]. The RAB had a significant increase in yellowness following pasteurisation, and this change in compounds could be related to the slight degradation of anthocyanin breakdown. A chromatographic analysis of the components responsible for the antioxidant activities would prove useful in the future to assess how pasteurisation might have led to changes in the structures of the compounds and affected the antioxidant activity of both beverages.

Colour differences between the two beverages were also observed, with RAB initially being darker and less yellow than SSB. Pasteurisation led to a slight increase in yellowness in RAB, but these changes were not visually perceptible, and both beverages retained an appealing pink colour. There is ed literature evaluating how Reb-A influences colour stability compared to sugar during pasteurisation, potentially being involved in interactions with other components in the juices and anthocyanin stability which require further studying. A desirable pink colour was achieved in the product development, aligning with both social media and consumer demands for more striking colours, and visually appealing food products [109-111]. Similar findings were reported by [112], where pasteurisation at 80-99°C increased L* values in a stevia-sweetened exotic fruit beverage and the b* values increased with thermal treatment independent of stevia glycosides addition. These results were attributed to the partial precipitation of suspended unstable particles in the fruit juice, which could explain the observed results in this study [112].

Sensory analysis revealed a clear consumer preference for SSB, particularly among male participants, who are reported to favour sugary beverages over healthy options [86]. However, RAB still received high acceptability scores, especially among female participants, and was rated above 6 on a 9-point scale by most consumers. This positive reception of RAB contrasts with earlier reports of negative consumer responses to stevia-sweetened beverages, likely due to the formulation's use of lemon and inulin, which help mask any potential bitterness or aftertaste associated with stevia [30,49,113].

A consumer-based panel is important for gaining consumer perspective and product acceptability; however, consumers lack knowledge on specific sensory attributes, and variability in scores is expected [114,115]. Trained assessors are recommended in new product development to accurately characterise the sensory properties of food, as they have an understanding of sensory attributes [115]. Although consumers offer valuable insights, the variability outlines the need for trained assessors to confirm the consumer’s findings for a more comprehensive understanding of the product’s sensory attributes and its potential market success.

Overall, replacing sugar with Reb-A in this beverage formulation resulted in a product with enhanced antioxidant potential and good consumer acceptability, particularly when combined with flavour-masking ingredients. While SSB remains the preferred choice for some consumers, RAB offers a promising, lower-calorie alternative that aligns with current trends toward healthier beverage options, without compromising on taste or functional benefits. Pasteurisation had only a minor impact on the comparative results, further supporting the suitability of both sweeteners in thermally processed functional beverages.

CONCLUSION

This study displayed the potential of using the natural sweetener Rebaudioside-A to produce a functional beverage, addressing the market demand for healthier alternatives free from artificial additives. Sensory analysis revealed a greater preference and acceptability for the sugar beverage; however, the Rebaudioside-A beverage received positive sensory scores, a good acceptability index, highlighting its market potential. This study highlighted men’s preference for sweetness, as they significantly preferred the sugar beverage, whereas females showed no significant preference, therefore indicating acceptance of the Rebaudioside-A beverage. Future *in vivo* trials would provide a comprehensive insight into whether the bioavailability of antioxidants demonstrates positive health effects to consumers. Overall, the development of an all-natural plant-based functional beverage using Rebaudioside-A displays potential for success within the Irish food market, providing consumers with a beverage rich in bioactive compounds and prebiotic fibres.

List of abbreviations: T2DM, Type 2 Diabetes Mellitus; CVDs, Cardiovascular Diseases :SSB, Sugar Sweetened Beverage; RAB, Reb-A Sweetened Beverage; Reb-A, Rebaudioside A; FB, Functional Beverage; ADI , Acceptable Daily Intake ; TSS , Total Soluble Solids; FRAP, Ferric Reducing Antioxidant potential; AAE , Ascorbic Acid Equivalents; TPC, Total Polyphenolic content; GAE, Gallic Acid Equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TAC, Total Monomeric Anthocyanin content; AI, Acceptability Index; ANOVA, analysis of variance; SD, Standard deviation; CV, Coefficient of Variation; RDI, Recommended Daily Intake.

Authors' contributions: FHS conceptualized the research topic and served as the lead scientist. LS and MW developed the beverage formulation and performed the physical, chemical, and sensory analyses and development of the manuscript. LS conducted the statistical analyses. FHS and DG provided detailed guidance and critical review of the manuscript, advised on the writing process, and contributed to the results discussions and manuscript preparation. All authors reviewed and approved the final version of the article.

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