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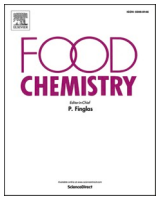

Influence of sugar composition in yoghurt on sweet receptor activation and sensory sweetness perception

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ABSTRACT

Sweetness is sensed by sweet taste receptor heterodimer (TAS1R2/TAS1R3) which responds to sucrose, glucose, fructose, and galactose. Limiting added sugar in dairy foods while maintaining sweetness for palatability is important in healthy food design. We investigated the influence of sugar composition in yoghurt on sweet receptor activation and on subsequent sensory sweetness perception. We found that sweet receptor activation was enhanced by the addition of sucrose post-fermentation to Yoflex® yoghurts (CH-1 *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and YF-L811 *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*). Sensory evaluation by trained panellists supported these findings. Analytical profiling revealed variability in residual sugar composition, particularly galactose. Receptor assays revealed a novel potentiating effect of galactose on sucrose-induced receptor activation. Sugar type and composition are key determinants of yoghurt sweetness and can be predicted by *in vitro* sweet receptor assays to determine sugar synergies as a strategy for minimising added sugars in dairy products.

1. Introduction

Increasing consumer demand for low-sugar fermented dairy products has prompted extensive research into compositions that enhance sweetness perception without excessive sugar addition (McCain et al., 2018; Wan et al., 2021). In yoghurt, sweetness is primarily derived from sugar molecules such as sucrose, glucose, lactose, and galactose (Sørensen et al., 2016; Wan et al., 2021). During yoghurt fermentation, lactic acid bacteria (LAB), typically *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* hydrolyse lactose into glucose and galactose via β-galactosidase. Glucose is converted to lactic acid, lowering the pH, and this transforms milk into yoghurt by promoting protein coagulation (Bintsis, 2018). Galactose, in contrast, may be metabolised or remain unchanged, depending on the strain's metabolic capacity (Sørensen et al., 2016).

The shift in sugar composition during fermentation influences not only the nutritional profile of yoghurt but also its perceived sweetness. Different LAB strains produce varying levels of residual sugars and acidity, which in turn can affect sweetness perception. Sucrose and glucose are known to strongly activate the human sweet taste receptor TAS1R2/TAS1R3 (Ahmad & Dalziel, 2020; Nelson et al., 2001), while galactose is less sweet in human sensory studies and remains relatively understudied in human receptor assays. It is unknown whether galactose is less potent or efficacious in activating the receptor.

Sweetness in yoghurt is shaped by complex receptor-level interactions rather than the sum of individual sugars. Blends can enhance perception; for example, sucrose shows additivity with fructose and synergy with glucose (Schiffman et al., 1995), highlighting the potential of low-level sugar blends to boost sweetness.

Different ligands bind distinct domains of the TAS1R2/TAS1R3 heterodimer: sucrose, aspartame and saccharin act on the extracellular domain of TAS1R2, while cyclamate and neohesperidin dihydrochalcone (NHDC) interact with the TAS1R3 transmembrane domain (Fujiwara et al., 2012; Jang et al., 2021). These ligands can synergistically potentiate the receptor response. While most research has examined artificial sweeteners (Acevedo et al., 2018; Kemp et al., 2011), the potential for natural sugars in fermented dairy (glucose, galactose, lactose) to act synergistically or additively remains underexplored. Understanding these effects in fermented milk and their association with sensory perception is essential for optimising sweetness in reduced-sugar dairy products.

Recent mapping of the full TAS1R2/TAS1R3 3D molecular structure

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using cryo-electron microscopy (Juen et al., 2025; Shi et al., 2025) and a rapid receptor-based FLIPR (fluorescence imaging plate reader) assay to measure the response to ligand binding are tools for advancing molecular taste prediction of sweetness. However, the link between microbial metabolism, sugar synergy, and sweet receptor activation remains poorly understood.

Human sensory evaluation and *in vitro* sweet taste receptor assays are complementary tools for assessing sweetness. While sensory tests can be affected by individual variability, receptor-based assays using the TAS1R2/TAS1R3 complex offer an objective, mechanistic measure of sweetness, making them valuable for preliminary screening and molecular insight prior to sensory validation.

This study aimed to address this gap through two key objectives: (1) to assess whether the sweet taste receptor can differentiate between high added sugar and natural low sugar content following fermentation, and (2) to investigate how microbial sugar metabolism during fermentation alters sugar composition and influences receptor activation. This integrated approach linked sensory analysis with molecular insights to deepen our understanding of sweetness perception and establish a rapid, cost-effective method for evaluating the sweetness of final products.

To achieve this, we used an *in vitro* FLIPR assay to assess the activation of TAS1R2/TAS1R3 by different yoghurt samples prepared with commercial *Lactobacillus* strains, CH-1 and YF-L811, with and without sucrose supplementation, and commercial yoghurt products, a yoghurt with added sugar and a non-sweetened yoghurt kefir product. Receptor response was correlated with sweetness scores determined using a trained sensory panel.

2. Methods

2.1. Preparation of yoghurt

Skim milk (SM) powder (Fonterra Co-operative Group, New Zealand) was reconstituted with water to give a solution with 3% protein and placed overnight at 4 °C for full rehydration. Yoghurt was prepared by inoculating reconstituted SM with 5% of an inoculum of either CH-1 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) (CH1) or YF-L811 (*Lactobacillus rhamnosus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) (YFL) (both purchased from Chr. Hansen, Denmark). Additionally, a commercial kefir ('The Collective', NZ) (Kefir) and Ambpomial Greek yoghurt (Yili, China) (AGY) were purchased from a local Asian supermarket. For the CH1 and YFL yoghurts, 8% sucrose and 0.25% pectin were added. The inoculated SM was incubated at 43 °C until a pH of 4.5 was reached (Table 1).

To enable the addition of the yoghurts to the cell layer for the sweet receptor assay, yoghurt samples were centrifuged at 10,000 ×*g* for 45 min at 4 °C to remove solids as reported elsewhere (Ahmad et al., 2023; Maes et al., 2025). They were then filtered through a 10 kDal cutoff remove proteins and large peptides. They were diluted 1/5 into a physiological solution at pH 7.4 that was ionically balanced (including calcium) for cell assay compatibility. The resulting clear supernatants, containing soluble sugars, were carefully collected and aliquoted. Samples were then stored at −20 °C until used in the receptor assay. These supernatants were serially diluted in the assay buffer and applied to the sweet receptor-expressing cell lines to assess receptor activation as an increase in intracellular calcium flux.

Table 1
Yoghurts used in receptor assay and sensory study.

Sample	Product	Protein %	Fat %	CHO %	8% Sugar added
CH1	Experimental yoghurt	3.5	0.2	4.1	
CH1+	Experimental yoghurt*	3.4	0.1	12.8	+
YFL	Experimental yoghurt	3.4	0.1	4.2	
YFL+	Experimental yoghurt*	3.5	0.1	13.0	+
Kefir	'The Collective', New Zealand	3.7	2.0	3.8	
AGY	Ambpomial (Greek), Yili, China	3.1	3.3	12.0	

* 8% *w*/w sugar (Woolworths, NZ Brand pure cane white sugar) and 0.25% w/w pectin (Grindsted® Pectin SY 200) added. CHO: carbohydrate. Composition data from the product information sheet (Dupas de Matos, Chen, Maggs, Godfrey, & Hort, 2025).

2.2. Sugar analysis

Sugar concentrations in yoghurt samples were determined using Enzytec™ Liquid enzymatic kits (R-Biopharm AG, Germany), following the manufacturer's instructions. Samples were centrifuged at 10,000 ×*g* for 10 min at 4 °C, and the supernatants were appropriately diluted. Absorbance was measured at 340 nm, and sugar concentrations were calculated using standard curves. The standard concentration–response curves for all four sugars are provided as Supplementary Fig. S1. Results are expressed as millimolar (mM).

2.3. Sensory evaluation

2.3.1. Ethics statement

The Massey University Human Ethics Committee assessed the sensory study, which was considered low risk, and gave ethical permission (Human Ethics Notification approval number: 4000018821). Prior to attendance, panellists received an information sheet outlining the details and provided informed written consent. Appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of this research according to legal requirements (Privacy Act 2020, NZ). For example, participants were assigned a unique code to ensure anonymity. Upon completion of each session, participants were offered a supermarket voucher and a treat as compensation for their time.

2.3.2. Panel training

Perceived sample sweetness was determined using a Modified Quantitative Descriptive Analysis approach undertaken to profile a range of yoghurts as part of a larger study. A panel ($n = 9$) were trained to evaluate unflavoured yoghurt following ISO 22935-1 (ISO, 2009). To generate sensory descriptors, different yoghurts ($n = 13$) were presented to the panel and following discussion they agreed upon 19 sensory attributes, including sweetness, to differentiate between the yoghurts. The panel were trained to measure all attributes across more than 55 sessions lasting ~110 h in total.

2.3.3. Sweetness evaluation

All yoghurt samples (50 g/cup) were stirred and served according to a Williams Latin square design (in duplicate) in 90 mL odour-free lidded plastic cups coded with a random 3-digit number (Kemp et al., 2011). Panellists were instructed to stir each sample before tasting using a straw. Plain crackers (Arnott's Biscuits, NSW, Australia) and filtered water were provided as palate cleansers. Sensory evaluation took place in sensory booths at the Feast (Food Experience and Sensory Testing) Laboratory under red lights to minimise appearance differences. Attributes, including sweetness, were scored on a continuous line scale (from 'not very' to 'very') converted to 0–10 for data analysis.

2.4. FLIPR assay for sweet taste receptor activation

Sweet receptor activation was assessed *via* intracellular calcium mobilisation using a Fluorescent Imaging Plate Reader assay kit (FLIPR, Molecular Devices, USA) as previously described (Ahmad et al., 2023; Maes et al., 2025). Cells at 70–80% confluency were washed with Hanks' Balanced Salt solution loaded with a fluorescent calcium indicator dye (Molecular Devices, USA), and incubated for 1.5 h at 37 °C, 5% CO_2. The sugar ligands and the yoghurt samples were added using a

FlexStation®3 Microplate Reader, and fluorescence was recorded at 485 nm excitation and 525 nm emission over 1.54 s.

The yoghurt samples were diluted in two-fold dilution factors from undiluted stock, ranging from 1-fold down to a 0.007 factor dilution (Fig. 1), and added to both receptor-expressing and control cell lines.

Four independent experiments were typically carried out from separate cell grow ups on different days which were repeated across 3 wells per plate, giving a total of 12 wells. Thus 12 points per concentration were then plotted as relative fluorescent units (ΔRFU = maximum RFU - minimum RFU). EC_{50} of a 4-parameter dose response curve was calculated in Prism version 9 (GraphPad Software Inc., CA, USA) using nonlinear regression, "Y = Bottom + (Top-Bottom)/(1 + 10^((LogEC$_{50}$-X)*HillSlope))" where X and Y represent two axis of the curve.

2.4.1. Inducible stable expression system for human sweet taste receptor heterodimer

The stable cell line for human sweet taste receptor heterodimer (TAS1R2/TAS1R3) was generated by using lentivirus vector system in tetracycline- Tet-On 3G system (Takara Bio Inc., Japan) and CHO-K1-Gα15 as a parent cell line (#M00257; Genscript, USA). The Tet-On 3G-293 cells were used as lentiviral packaging cells and transfected with 20 μg of plasmid DNA in 10-cm dish. Subsequently, supernatants were harvested and concentrated, and the virus titer were determined with Lenti-X p24 Rapid Titer Kit (Takara Bio, Japan). The target cells, CHO-K1-Gα15 cells were grown in Ham's F12K medium supplemented with 10% foetal serum and viral co-infections were done in the presence of Polybrene (8 μg/mL; Merck, USA) with appropriate multiplicity of infection and further refed with growth medium 48 h later. The stable cell pool expressing TAS1R2/TAS1R3 was selected and maintained by puromycin and G418. Subsequently, relative expression of the transcript was evaluated by RT-qPCR, obtaining a high expression level in the presence of doxycycline (1 μg/mL). Cells were cultured in Ham's F-12 K (Kaighn's) Medium (#21127022, Gibco, TX, USA) supplemented with 10% FBS, penicillin-streptomycin 1% and selection antibiotics (G418 (800 μg/mL), Hygromycin B (200 μg/mL), Puromycin (10 μg/mL) & Blasticidin (6 μg/mL)). Parental CHO-K1-Gα15 cells, which lacked TAS1R2/TAS1R3, served as a negative control to confirm receptor-specific activation.

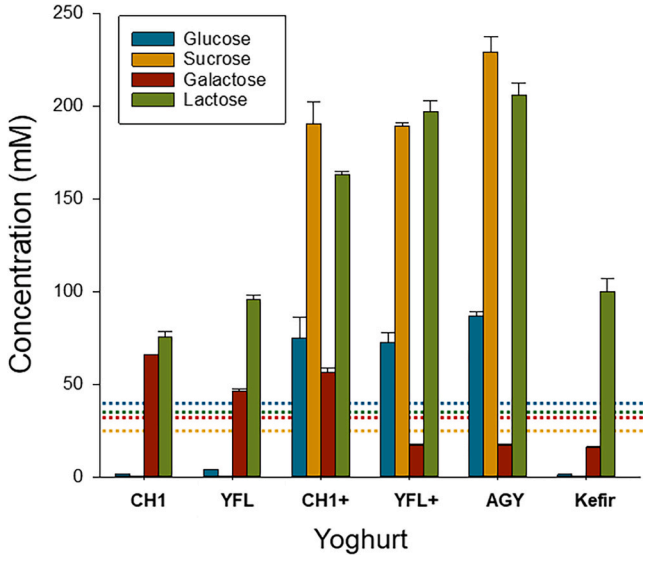

Fig. 1. Sugar composition of yoghurt samples, including CH1-fermented milk, YFL-fermented milk with and without 8% added sucrose (+), and commercial yoghurt brands (AGY) and kefir. Data are presented as mean ± SE ($n = 5$). Note: galactose is highest in CH1 ($p < 0.05$, one-way ANOVA with Tukey's post-hoc test). Dotted lines (as per colour key) represent human sensory recognition thresholds (Carocho et al., 2017; Robyt, 1998). Also refer to Supplementary Table.

2.5. Statistical analysis

Sweet receptor activation responses were expressed as ΔRFU (relative fluorescence units), calculated as the difference between maximum and minimum fluorescence values. Data were averaged from three technical replicates, with experiments conducted in three biological replicates. EC_{50} values were determined using nonlinear regression analysis using four parametric logistic model in GraphPad Prism 10.5 version. The relative comparisons were done using one-way ANOVA followed either by Tukey's or Dunn's multiple comparison test, with statistical significance set at $p < 0.05$. Perceived differences in sweetness were determined using one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

3. Results and discussion

3.1. Sugar composition of yoghurt samples and sweet receptor activation

To understand sweetness perception in yoghurts, we first analysed the sugar composition of the experimental and commercial samples (Fig. 1 & Supplementary Table). Lactose was the predominant sugar in all yoghurts, consistent with their dairy origin. Among the experimental cultures (CH1 and YFL), residual galactose levels varied markedly: CH1 contained ~42% more galactose than YFL, while glucose concentrations were slightly higher in YFL. Both experimental yoghurts contained negligible sucrose (<0.1 mM) prior to supplementation. When 8% sucrose was added (CH1+ and YFL+), sucrose concentrations increased to ~190 mM, a level similar to that in the commercial sweet yoghurt (AGY). Kefir, by contrast, contained low concentrations of sucrose and galactose. These compositional differences provided the basis for evaluating sweet receptor activation.

We then compared the ability of these yoghurt samples to activate the sweet taste receptor heterodimer (TAS1R2/TAS1R3) using CHO-K1 cells expressing the receptor (Fig. 2). The supernatants of the yoghurt samples were diluted (0.2–1.0 dilution factors) and added to the receptor assay to determine the concentration range that would elicit a response from the sweet receptor. When the experimental yoghurt samples were assessed, the receptor activation signal was the highest and consistent across yoghurts at 0.5 dilution factor (Fig. 2). The high sugar yoghurts with added sucrose (CH1+ and YFL+) showed stronger activation responses of the sweet taste receptor than their low sugar control counterparts (CH1 and YFL) at dilution factors 0.5 and 1.0.

For the two commercial products, AGY strongly activated the sweet receptor, similar to the level for CH1+ and YFL+. In contrast, kefir produced only a low level of activation, similar to CH1 and YFL yoghurts (no sugar added). Our results demonstrate that the sweet receptor was able to distinguish between high and low sugar content among different yoghurts in the 4–13% sugar range.

3.2. Sensory perception of yoghurts

Results from the trained sensory panel indicated that when supplemented with sucrose, both CH1+ and YFL+ cultures exhibited high sweetness ratings compared to their respective controls without sucrose addition (Fig. 3). Similarly, the commercial sweet yoghurt (AGY) displayed elevated sweetness ratings, consistent with its high sucrose content, whereas kefir exhibited minimal sweetness perception, in line with its low sucrose content.

3.3. Comparison of receptor detection with sensory perception for yoghurt sweetness

Our sweet receptor detection data for the yoghurts strongly

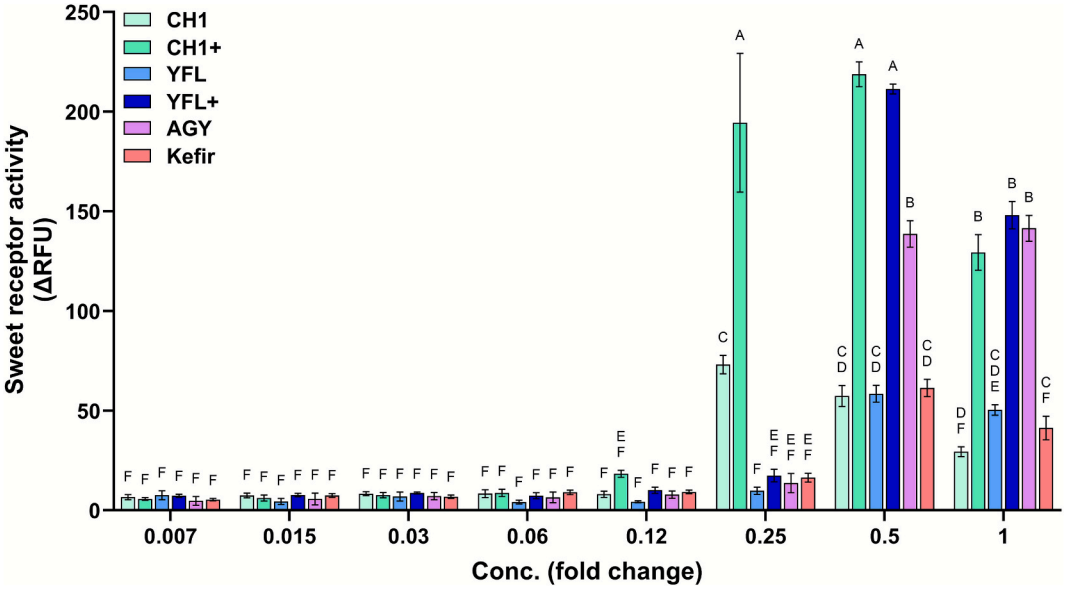

Fig. 2. Bar chart showing sweet receptor activity measured as relative fluorescent units (ΔRFU) of intracellular calcium levels in CHO-K1 cells stably expressing the sweet receptor. Responses are shown for yoghurt samples produced using the CH1 strain, YFL with and without 8% added sugar, and commercial yoghurt products. Data are presented as mean ± SE, four independent experiments with three replicates for each concentration ($n = 4$). Results annotated with different uppercase letters are significantly different ($p < 0.05$, one-way ANOVA with Tukey's post-hoc test).

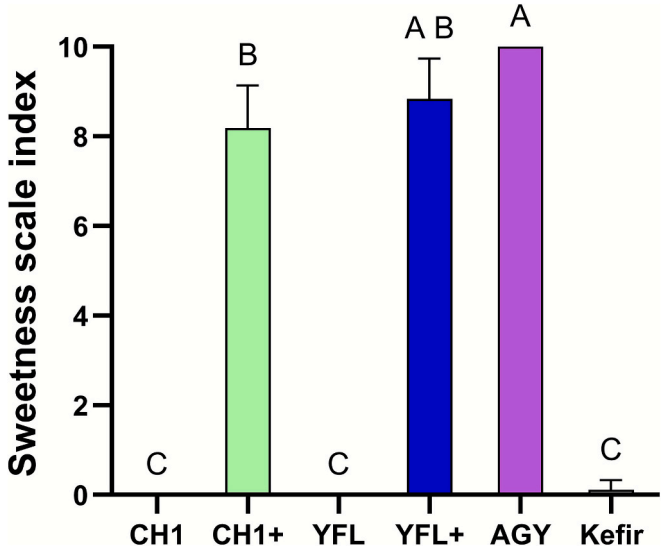

Fig. 3. Sweetness perception scores from a trained sensory panel ($n = 9$) trial evaluating fermented milk samples, including CH1-fermented milk, YFL-fermented milk with and without 8% sugar, and commercial yoghurt brands (Chinese brand AGY and kefir). Sweetness was rated on a scale from 0 (not very) to 10 (very) across two independent sessions. Data are presented as mean ± SD. Different letters (A, B, C) indicate statistically significant differences between groups ($p < 0.05$, one-way ANOVA test with Dunn's multiple comparison analysis).

associated with the trained sensory panel perception data. These findings support growing evidence that *in vitro* sweet taste receptor assays can predict human sensory perception of sweetness (Belloir et al., 2024). The heterologous expression system of the human TAS1R2/TAS1R3 receptor has been widely used to evaluate responses to a variety of sweeteners, including mono- and disaccharides (Belloir et al., 2024; Nelson et al., 2001; Nie et al., 2005). Our study extends these applications to fermented dairy systems, demonstrating that variations in sugar content achieved through controlled sucrose supplementation can modulate sweet receptor activation in a way that aligns with perceived sweetness. Moreover, previous studies have demonstrated that sweetness perception is influenced not only by sucrose concentration but also by interactions among sugars and with acids (Bonnans & Noble, 1993; Jang et al., 2021; Schiffman et al., 1995), which may help explain the observed differences between CH1 and YFL yoghurt samples. By linking receptor-based biochemical readouts with actual sensory panel data, this study strengthens the case for using sweet receptor assays as a practical and objective screening tool to differentiate dairy products with and without added sugar.

Beyond prediction of sensory outcomes, receptor-based assays offer distinct advantages for initial sweetness evaluation. Human sensory tests, though essential for perceptual validation, can be influenced by individual variability and psychological factors. In contrast, functional receptor assays using the TAS1R2/TAS1R3 complex provide an objective and mechanistic measure of sweetness potential, enabling reproducible, high-throughput screening of product formulations prior to sensory testing.

An exception to this overall alignment occurred when comparing CH1+ and AGY. At a 1× dilution, receptor activation was similar, but at 0.5–0.12× dilutions, CH1+ elicited stronger responses despite being perceived as less sweet. This discrepancy likely reflects the influence of other sensory modalities, such as texture, viscosity or acidity on sweetness perception. For example, trained panel studies indicate that acidity can strongly shape yoghurt flavour, while variations in viscosity in dairy products can suppress perceived sweetness even at equivalent sugar levels (Desai et al., 2013; Ott et al., 2000). These findings highlight that, although *in vitro* receptor assays provide valuable insight into the biochemical basis of sweetness, they represent only one component of the complex multisensory experience of taste.

3.4. Synergy among sweet receptor activators

In vitro sweet receptor assays revealed higher activation for CH1 yoghurt at a lower dilution (0.25) than YFL, implying differences in sugar composition (Fig. 1 & Supplementary Table). Both yoghurts contained residual galactose, with CH1 having ~42% more, which corresponded with the stronger receptor response at 0.2 dilution. The accumulation of galactose is common in fermented dairy due to the

galactose-negative (Gal^{-}) phenotype of some LAB strains, such as *Streptococcus thermophilus*, which preferentially metabolise glucose from lactose and excrete galactose (Hutkins & Morris, 1987). Since monosaccharides can enhance the perceived sweetness of sucrose (Jang et al., 2021; Schiffman et al., 1995), higher galactose levels may potentiate sucrose-induced receptor activation. To explore this, we investigated the potential contribution of the various sugars in the yoghurts to sweet receptor activation through an in-depth analysis of the concentration-dependence of the sweet receptor.

The concentration–response curve for sucrose overlaps with the activation range previously reported in HEK cells (Nelson et al., 2001; Nie et al., 2005). However, the observed EC_{50} for sucrose in this study was 190 ± 9 mM (Fig. 4A), which is higher than the values reported for TAS1R2/TAS1R3 expressed in HEK cell lines (40–100 mM) (Palmer et al., 2021; Servant et al., 2011). The higher EC_{50} observed here likely reflects differences in the cell line or experimental conditions used factors known to affect apparent ligand potency rather than an intrinsic change in receptor affinity (Xu et al., 2004).

Because humans sense sucrose as sweeter than glucose or galactose, we expected the receptor response to follow a similar pattern. As expected, the concentration-response analysis comparing the sweet receptor response to sucrose with that of glucose and galactose showed that sucrose produced the strongest receptor activation (Fig. 4A), with the lowest EC_{50} value (190 mM), followed by glucose (296 mM) (Fig. 4B). Galactose had a very steep slope, and little activation was detected at concentrations less than 316 mM (Fig. 4C). To understand whether galactose and sucrose might work synergistically together to activate the sweet receptor, we used a low concentration of galactose - insufficient on its own to activate the sweet receptor – in combination with increasing concentrations of sucrose (Fig. 5).

When we added 60 mM galactose with sucrose, we detected a leftward shift (Fig. 5A), with an approximate 36% reduction in EC_{50} ($p < 0.01$) (Fig. 5B). This indicated that galactose enhanced the apparent affinity of the sweet taste receptor for sucrose. This provides evidence of a synergistic interaction between these sugars in enhancing sweetness at the receptor level. These results suggest that residual galactose content, shaped by fermentation bacterial strain and metabolic phenotype, may be used to modulate receptor-level sweetness. The human TAS1R2/TAS1R3 sweet taste receptor is a class C GPCR with large extracellular Venus Flytrap Domains (VFTs) in TAS1R2 that bind sweet ligands, including sucrose (Juen et al., 2025; Shi et al., 2025). While sucrose binds the VFT to stabilize the active receptor conformation, galactose alone is a weak agonist. When combined with sucrose, galactose may act as a positive allosteric modulator, stabilizing the sucrose-bound active state or subtly enhancing cooperativity between subunits, which could explain the observed leftward EC_{50} shift. Although this provides a plausible mechanistic hypothesis, the exact binding site and molecular interactions for galactose remain to be determined. The concentration range of sucrose used in the receptor assay (0.1–600 mM) is comparable to the threshold for detection in the general human population (12 mM) and overlaps with that in our high sugar yoghurts (189–229 mM).

Our findings demonstrate that both the type and concentration of sugars, as well as the residual sugars resulting from fermentation, can influence sweet taste receptor activation. The observed synergy between sucrose and galactose provides mechanistic support for the notion that both concentration and relative potency modulate sweetness perception, as previously reported in human sensory studies (Keast & Breslin, 2003; Schiffman et al., 1995; Stone & Oliver, 1969). This highlights the potential to optimise sweetness perception in fermented dairy products through careful control of sugar composition and fermentation processes.

Future studies should consider the impact of other fermentative microorganisms and their sugar utilisation profiles to further elucidate how microbial communities shape sweetness in fermented foods. However, this should be done in conjunction with sensory taste panel studies using experimental yoghurts with known composition.

Our finding that galactose can potentiate the sucrose response at the receptor level is new. It was known that sucrose and glucose can act in synergy where combining these enhanced the human perception response by 20–30% (Schiffman et al., 1995; Stone & Oliver, 1969). Many other sweetener combinations show synergy at the level of perception and recent *in silico* simulation studies predict sugar synergy as a key mechanism in sweetener activation of TAS1R2/R3 receptors. While there is no high resolution 3D molecular structure determined yet with natural sugars bound, *in silico* molecular docking and functional TAS1R2/R3 assays show that sucrose binds to a specific extracellular site on TAS1R2 (Jang et al., 2021; Shi et al., 2025) and TAS1R3 is important for receptor stability in the membrane (Juen et al., 2025). This will also shed light on binding locations and conformational changes responsible for sugar synergies.

A limitation of this study is that while providing mechanistic insight into receptor activation by different sugars, the *in vitro* sweet taste receptor assay does not fully replicate the complexity of human taste perception. Factors such as the food matrix, saliva composition, oral processing, and interactions with other sensory modalities are not captured by the assay. Therefore, receptor activation results should be

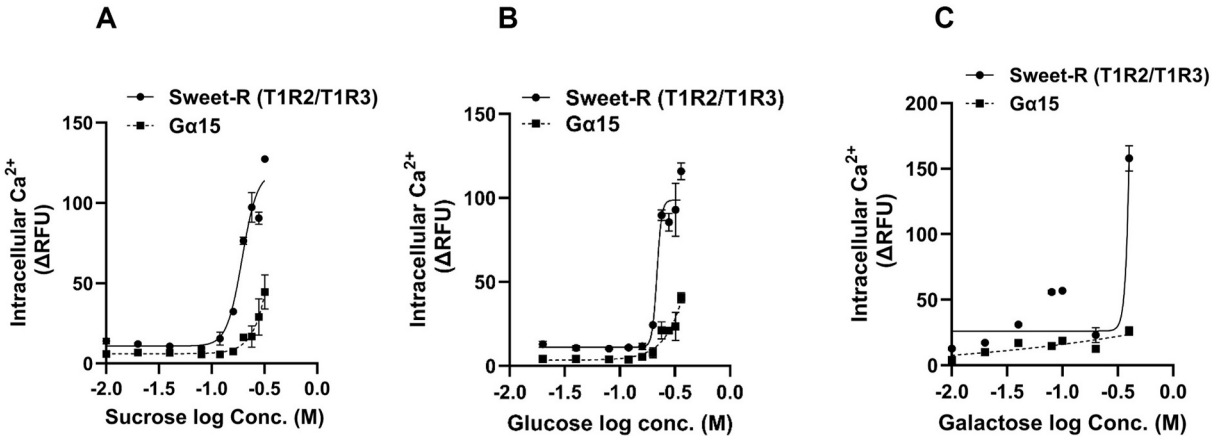

Fig. 4. Dose-response curves showing sweet receptor activity measured as relative fluorescent units (ΔRFU) of intracellular calcium levels in response to sucrose (95% confidence interval (CI); Hill slope- 3.1 to 11.7; R^2–0.96) (A), glucose (95% CI, Hill slope- 9.9 to undefined, R^2–0.96) (B), and galactose (95% CI, Hill slope- -1.16 to undefined, R^2–0.84) (C) in CHO-K1 cells stably expressing the sweet receptor and CHO-K1 cells stably expressing Gα15 (G15) as a control. Data are presented as mean ± SE with four independent experiments with three technical replicates for each concentration ($n = 4$). EC_{50} values are: sucrose, 190 mM ± 9; and glucose, 296 mM ± 81.

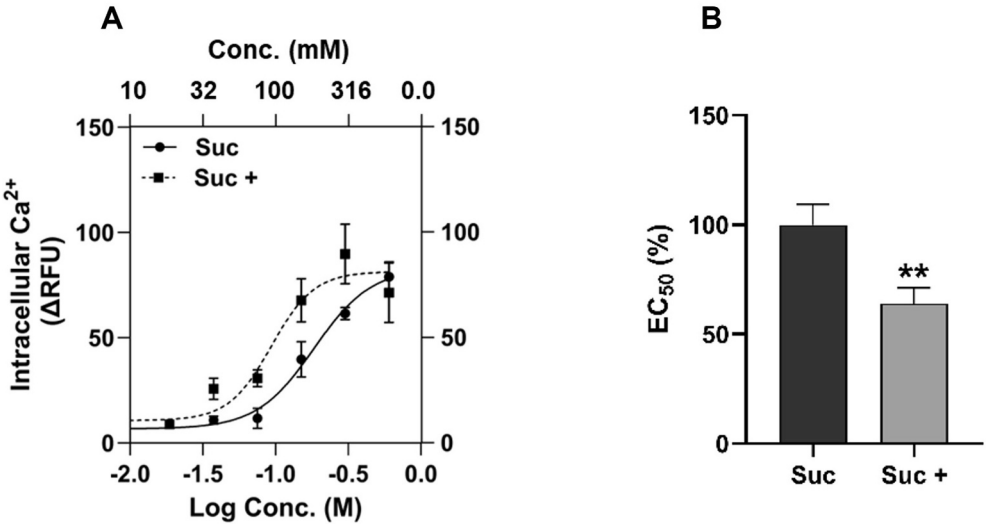

Fig. 5. Dose–response curves and EC_{50} comparison illustrating sweet receptor activity, measured as relative fluorescence units (ΔRFU) of intracellular calcium levels, in response to sucrose alone (Suc) and in combination with 60 mM galactose (Suc +) in CHO-K1 cells stably expressing the sweet taste receptor. (A) Concentration-response curve for sucrose alone and sucrose +. A leftward shift was observed in presence of galactose (95% CI, Hill slope- 1.9 to 3.4 and $R^2 = 0.98$ for sucrose; 95% CI, Hill slope 1.1 and $R^2 = 0.90$ for sucrose +). EC_{50} values: sucrose = 185 ± 17 mM; sucrose + galactose = 118 ± 13 mM. (B) Bar graph showing the EC_{50} values (expressed as %) for sucrose alone and sucrose +, indicating a reduction in EC_{50} upon galactose addition. Data are presented as mean ± SE with three independent experiments with three technical replicates for each concentration ($n = 3$). Statistical comparison by unpaired *t*-test: $^{**}p < 0.01$.

interpreted as complementary to sensory evaluation rather than a direct proxy for perceived sweetness.

4. Conclusion

We report new insights into the molecular mechanisms of sweetness detection, showing that both sugar type and concentration influence sweet receptor activation. We provide evidence that galactose acts synergistically with sucrose on TAS1R2/TAS1R3 receptors, potentiating the sucrose sweet response. Our study demonstrates that *in vitro* cell-based assays complement sensory evaluations, offering mechanistic insights into how different sugar compositions modulate overall sweet receptor activity. Future research should investigate interactions among sugars and assess diverse fermentative microorganisms to understand better how microbial communities shape sweetness in fermented foods.

CRediT authorship contribution statement

Raise Ahmad: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Amanda Dupas de Matos:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Joanne Hort:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Li Day:** Writing – review & editing, Project administration, Funding acquisition. **Julie E. Dalziel:** Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.148351.

Data availability

Data will be made available on request.

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