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LITERATURE REVIEW:

Use of Ice Cream Containing Probiotic an Effective Way to Control Dental Caries?

¿El uso de helados que contienen probióticos es una forma eficaz de controlar la caries dental?

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ABSTRACT: To perform a systematic review to assess the decrease in bacterial counts of *Streptococcus mutans* in patients who used ice cream enriched with probiotics. Is registered in the PROSPERO platform, by the code CRD42022371298. The databases were Scopus, Web of Science, Embase, Pubmed, Medline, and Science Direct, through a search algorithm based on the following PICO question: P-individuals systematically healthy and free of caries, I-enriched diet with probiotics, C-diet with ice cream without probiotics, O-*S. mutans* count after the diet, being only original articles. After the inclusion of metadata in the Rayyan platform, the text were read by two independent researchers, with inter-examiner kappa >0.9, with a final sample of 5 articles. There was a significant reduction of *S. mutans*, with p-values between <0.001 and <0.005 for all studies analyzed, maintaining the decrease for up to 7 days, conserving it for 90 days after the diet protocol, but in this case without statistical difference. The use of ice cream containing probiotics to reduce the bacterial count of *S. mutans* is promising, but the ideal strain and the exact number of days for maintenance of the diet are still unknown.

KEYWORDS: *Bifidobacterium lactis* Bb-12; *Lactobacillus acidophilus*; Oral biofilm; Oral health; *Streptococcus mutans*; Oral Hygiene.

RESUMEN: Realizar una revisión sistemática para evaluar la disminución del recuento bacteriano de *Streptococcus mutans* en pacientes que utilizaron helados enriquecidos con probióticos. El estudio está registrado en la plataforma PROSPERO, con el código CRD42022371298. Las bases de datos utilizadas fueron Scopus, Web of Science, Embase, Pubmed, Medline y Science Direct, mediante un algoritmo de búsqueda basado en la siguiente pregunta PICO: P (individuos sistemáticamente sanos y libres de caries), I (dieta enriquecida con probióticos), C (dieta con helado sin probióticos), O (recuento de *S. mutans* tras la dieta), siendo solo artículos originales. Tras la inclusión de metadatos en la plataforma Rayyan, los textos fueron leídos por dos investigadores independientes con un índice de kappa inter-examinador >0,9, con una muestra final de 5 artículos. Se observó una reducción significativa de *S. mutans*, con p-valores entre <0,001 y <0,005 en todos los estudios analizados, reducción que se mantuvo hasta 7 días y que se conservó durante 90 días después del protocolo de dieta, pero en este caso sin diferencia estadística. El uso de helados que contienen probióticos para reducir el recuento bacteriano de *S. mutans* es prometedor, pero aún se desconoce la cepa ideal y el número exacto de días para el mantenimiento de la dieta.

PALABRAS CLAVE: *Bifidobacterium lactis* Bb-12; *Lactobacillus acidophilus*; Biopelícula oral; Salud oral; *Streptococcus mutans*; Higiene buccal.

INTRODUCTION

Dental caries, ubiquitously acknowledged as the most widespread noncommunicable disease, afflicts over a third of the global population, with untreated cases surpassing 2 billion individuals worldwide (1). This pathology, predominantly caused by the bacterial agent *Streptococcus mutans*, leads to the degradation of dental tissues due to acidic by-products from bacterial metabolism (2). *S. mutans*, adept at colonizing the dental biofilm, metabolizes free sugars, particularly sucrose, from food and beverages into acids, progressively leading to tooth enamel demineralization (3). While mechanical removal of biofilm via toothbrushing is the cornerstone in caries management, alternative strategies have garnered research interest for comprehensive caries control (4).

Probiotics, defined as live microorganisms which, when administered in adequate amounts, confer a health benefit to the host (5), have emerged as a potential adjunct in dental health strategies. The spectrum of probiotics spans various genera, including but not limited to *Lactobacillus*, *Pediococcus*, *Propionibacterium*, and *Bifidobacterium*. These microorganisms, having established beneficial roles in diverse health conditions such as gut inflammation, hypercholesterolemia, and urogenital infections (6), are now being investigated for their efficacy in the oral health domain.

Clinical trials in dentistry have frequently employed probiotics from *Lactobacillus*, *Bifidobacterium*, *Streptococci*, and *Bacillus* genera (7). While the precise impact of these probiotics on dental caries is yet to be fully elucidated, it is hypothesized that they contribute to pathogenic biofilm disruption and modulate host defense mechanisms, including mucosal immune defense and macrophage activity (8). For efficacious caries prevention, probiotics must not only adhere to

and integrate into dental biofilms but also exhibit competitive attributes against cariogenic bacteria and a low-acid-producing metabolic profile (9).

Identifying an optimal vehicle for probiotic delivery remains an area of active research. Dairy products like yogurt, milk, and cheese are traditionally deemed suitable carriers. However, the widespread appeal and acceptance of ice cream, particularly among pediatric populations, present it as a novel and potentially effective medium for probiotic delivery (10,11). This systematic review, therefore, aims to critically evaluate the impact of probiotic-enriched ice cream on the reduction of *Streptococcus mutans* bacterial counts, a key indicator in dental caries prevention.

MATERIAL AND METHODS

PROTOCOL AND REGISTRATION

This systematic review follows all recommendations proposed by the PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses (12). The authors have registered this research on the PROSPERO (International Prospective Register of Systematic Reviews) platform under the CRD42022371298. The availability data may be request to the corresponding author (PHSS).

ELIGIBILITY CRITERIA

Clinical trials in which subjects were fed an ice cream enriched with a probiotic diet were included to analyze the salivary count of *Streptococcus mutans*. There was no restriction on the probiotic used to prepare the ice cream, year, or publication language. Other study designs than original articles and research without ice cream were excluded.

INFORMATION SOURCE AND SEARCH

The search strategy construction process was based on the PICO question: P (population) - systematically healthy individuals; I (intervention) - ice cream enriched with probiotic diet; C (comparison) - ice cream diet without probiotics; O (outcome)- salivary *S. mutans* count. The databases used for the search were Scopus, Web of Science, Embase, Pubmed, Medline, and Science Direct, conducted between October 25 and 30, 2022.

Thus, the search strategy was: (teeth or tooth or “dental caries” or “dental caries activity test” or “dental decay” or “decay, dental” “carious lesion”) and (probiotic or “dietary supplements” or synbiotics or prebiotics or “polysaccharides, bacterial” or lactobacillus or icrecream or “ice-cream” or “ice cream” or “cream, ice”) and (“colony forming units” or antibacterial or saliva or “saliva samples”).

SELECTION OF EVIDENCE SOURCES

The selection of the studies included in this review was performed by two independent blinded reviewers (MJFC and PHSS) using the Rayyan online platform - rayyan.qcri.org (13). The first step relied on eliminating duplicates and reading the titles, abstracts, and the whole paper. Inconsistencies were resolved in an agreement between the two examiners. The inter-examiner agreement was assessed by Cohen's Kappa test with a value of 0.92 (CI=0.91-0.93).

DATA CHARTING PROCESS

The information extracted from the articles (authors, year of publication, country of publication, sample, sample groups, time of analysis, probiotic, test used for salivary quantification of *S.*

mutans, and main results) was grouped, filled in, and checked by the two independent researchers (MJFC and PHSS) to examine possible inconsistencies and ensure data accuracy using Microsoft Office Excel 2016.

CRITICAL APPRAISAL OF INDIVIDUAL SOURCES OF EVIDENCE

The risk of bias analysis was performed using the RoB2 platform developed by Cochrane. The individual analysis of included articles was checked against six domains that briefly consider selection bias, performance bias, detection bias, attrition bias, reporting bias, and any other condition found. All articles included in this analysis were checked by two independent reviewers (MJFC and PHSS), and inconsistencies were resolved in an agreement between the two reviewers.

Regarding the quality of the scientific evidence, we used the GRADE (Grades of Recommendation, Assessment, Development and Evaluation) that evaluates the certainty assessment based on study design, risk of bias, inconsistency, indirect evidence, imprecision, and other considerations. The information was consolidated in the online software "GRADEPRO" by one of the researchers trained to manipulate the platform (MJFC) (14).

SYNTHESIS OF DATA

Data were analyzed using Revman 5.4.1. Data from the included studies were ordinal for the "Colony Formation Unit" outcome measure. Risk differences were used as an effect measure with 95% confidence intervals and a random effects model. We assessed heterogeneity using I^2 statistics. Data from some follow-up periods (1 hour, 7, 18, 30, 90, and 180 days) were included for the outcome.

RESULTS

We obtained 63 studies from all databases, in which 18 were duplicated. After screening, we included 6 articles in the final sample. The Figure 1 show the detailed selection process following PRISMA flow diagram.

We excluded seven studies that met eligibility criteria: six studies presented only *in vitro* approach (14-19), one study use yogurt rather than ice-cream as “vehicle” (20).

STUDIES CHARACTERISTICS

According to the obtained results, probiotic containing ice cream reduced *S. mutans* salivary load (CFU) in all the primary studies of this systematic review. The included articles used *Bifidobacterium lactis* Bb-12 (n=5; 83.3%) (21-25) combined or not with *Lactobacillus acidophilus* La-5 (n=3; 50.0%) (22-24), while only one study did not present the used probiotic (26). The studies were conducted in India (n=5; 83.3%) (22-26) or Türkiye (n=1; 16.7%) (21). In the selected studies, the sample range from 20 up to 60 participants, with a media of 37 individuals. The evaluation time ranged from 1 hour (26) up to 180 days (23). Five studies were published in the years 2010-2019 (n=83.3%) (22-26) and one was conducted before 2010 (21). The detailed information can be consulted in Table 1.

RISK OF BIAS IN STUDIES

The studies were very similar in the classification of the proposed tool. Four studies presented low risk of bias for all domains (23-26). Other 2 studies did not present all results, as media and standard deviation (21,22). According to the proposed protocol and the criteria previously explained in the material and methods section

(2.2), all studies were classified as having a low risk of bias. Detailed information of each study can be visualized in Table 2.

In this study, we also evaluated the evidence quality using the GRADE. All the 6 included studies were Randomized Clinical Trials (RCT), with non-severe problems about risk of bias, inconsistency, indirect evidence, and imprecision. Thus, we consider the evidence quality certainty as high.

RESULTS OF SYNTHESES

DESCRIPTIVE ANALYSIS

We included in the meta-analysis four studies that reported the CFU (media and standard deviation) in different periods. The difference in the number of studies in each follow-up is attributed to whether the outcome was reported by the authors. The follow-up of 18, 30, 90, and 180 days presented only one study each.

META-ANALYSIS

The risk difference for the comparison between ice-cream containing probiotics and ice-cream without probiotics for the 1-hour follow-up period was -50.01 with 95% CI between (-158.66, 58.64), with no statistically significant difference (p=0.370). The 7-days follow-up risk difference was -70.77 with CI between (-118.42, -23.12), with statistically significant difference (p=0.004). Overall heterogeneity was high with I^2>75%. Taking all the results together, the risk difference was -40.85 (-59.77, -21.93), with a statistically significant difference (p<0.0001) (Figure 2).

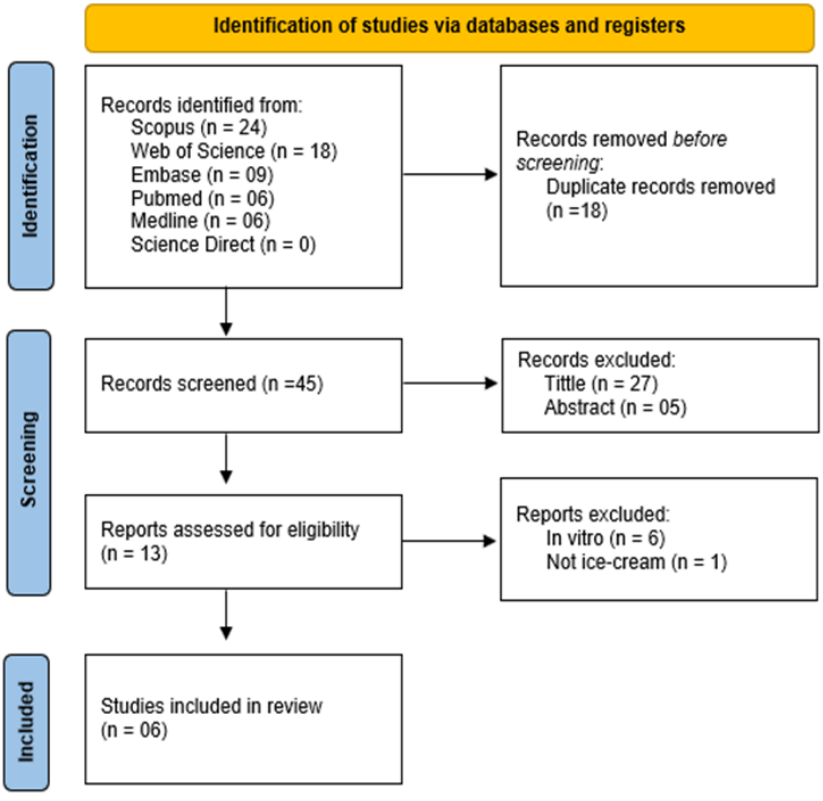

Figure 1. Search flowchart according to the PRISMA Statement.

Table 1. Analysis of articles included in the systematic review.

Study	Local	Sample	Groups	Time	Probiotic	Test	Results
Ashwin[24]	India	60 children (6-12 years)	G1: probiotic (n=30); G2: placebo (n=30)	7, 30 and 180 days	*Bifidobacterium lactis* Bb-12; *Lactobacillus acidophilus* La-5	CFU	Significant reduction in *S. mutans* count after seven days of ice cream ingestion (p<0.001) and also after 30 days of the washout period (p<0.001). After 180 days of the study period in both groups, the salivary levels of G1 were similar to the baseline. There was no significant reduction (p=0.076) by regular ice cream consumption.
Mahantesha[28]	India	50 children (6-12 years)	G1: probiotics in ice-cream (n=25); G2: probiotics in drink (n=25)	7 and 90 days	Ice-cream: *Bifidobacterium lactis* Bb-12; *Lactobacillus acidophilus* La-5	CFU	There is a significant reduction in the salivary level of GI in both groups after seven days (p<0.001). However, only the probiotic ice cream showed a reduction after the washout period, while the drink did not. Furthermore, there was no significant difference between probiotic ice cream and beverage.
Chinnappa[28]	India	20 children (12-14 years)	G1: probiotic (n=10); G2: placebo (n=10)	1 hou and 7 days	Drink: *Lactobacillus casei*	CFU	The study revealed a reduction in the salivary *S. mutans* count after 1 hour in all groups. However, after seven days, probiotic ice cream showed a statistically significant (p<0.001) reduction in *S. mutans* counts compared to the control.
Singh[28]	India	40 children (12-14 years)	G1: probiotic (n=20); G2: placebo (n=20)	7 and 14 days	*Bifidobacterium lactis* Bb-12; *Lactobacillus acidophilus* La-5	CFU	In the statistical analysis, it was observed that the probiotic ice cream promoted a statistically significant reduction (p-value=0.003) in the salivary levels of *S. mutans*, with no significant effect on the levels of *lactobacilli*.
Çaglar[25]	Türkiye	24 individuals	G1: probiotic (n=12); G2: placebo (n=12)	10 and 20 days	*Bifidobacterium lactis* Bb-12	CFU	There was a statistically significant (p<0.05) reduction in salivary *streptococci mutans* after the probiotic ice cream consumption. A decline in streptococci mutans counts was also observed after ingestion of the control ice cream, but the difference from baseline was not statistically significant. Salivary *lactobacilli* levels remained unchanged after both regimens.
Nagarajappa[27]	India	30 individuals	G1: probiotic (n=15); G2: placebo (n=15)	1 hour and 18 days	*Bifidobacterium lactis* Bb-12	CFU	A statistically significant reduction (p<0.05) of *S. mutans* was recorded in the test group. Even in the comparison between baseline and 18-day follow-up, there was a statistically significant difference (p<0.001). The same was not observed in the control group at any time analyzed.

CFU: Colony Forming Unit.

Table 2. Risk of bias assessment.

Study	Domain 1	Domain 2	Domain 3	Domain 4	Domain 5	Domain 6
Ashwin *et al.*[24]	-	-	-	-	-	-
Çaglar *et al.*[25]	-	-	-	?	-	-
Chinnappa *et al.*[29]	-	-	-	-	-	-
Mahantesha *et al.*[26]	-	-	-	-	-	-
Nagarajappa *et al.*[27]	-	-	-	-	-	-
Singh *et al.*[28]	-	-	-	?	-	-

+: High risk ok bias; -: Low risk of bias; ?: Not founded. Domain 1: risk of bias arising from the randomization process; Domain 2: effect of assignment to intervention; Domain 3: effect of adhering to intervention; Domain 4: missing outcome data; Domain 5: risk of bias in measurement of the outcome; Domain 6: risk of bias in selection of the reported result.

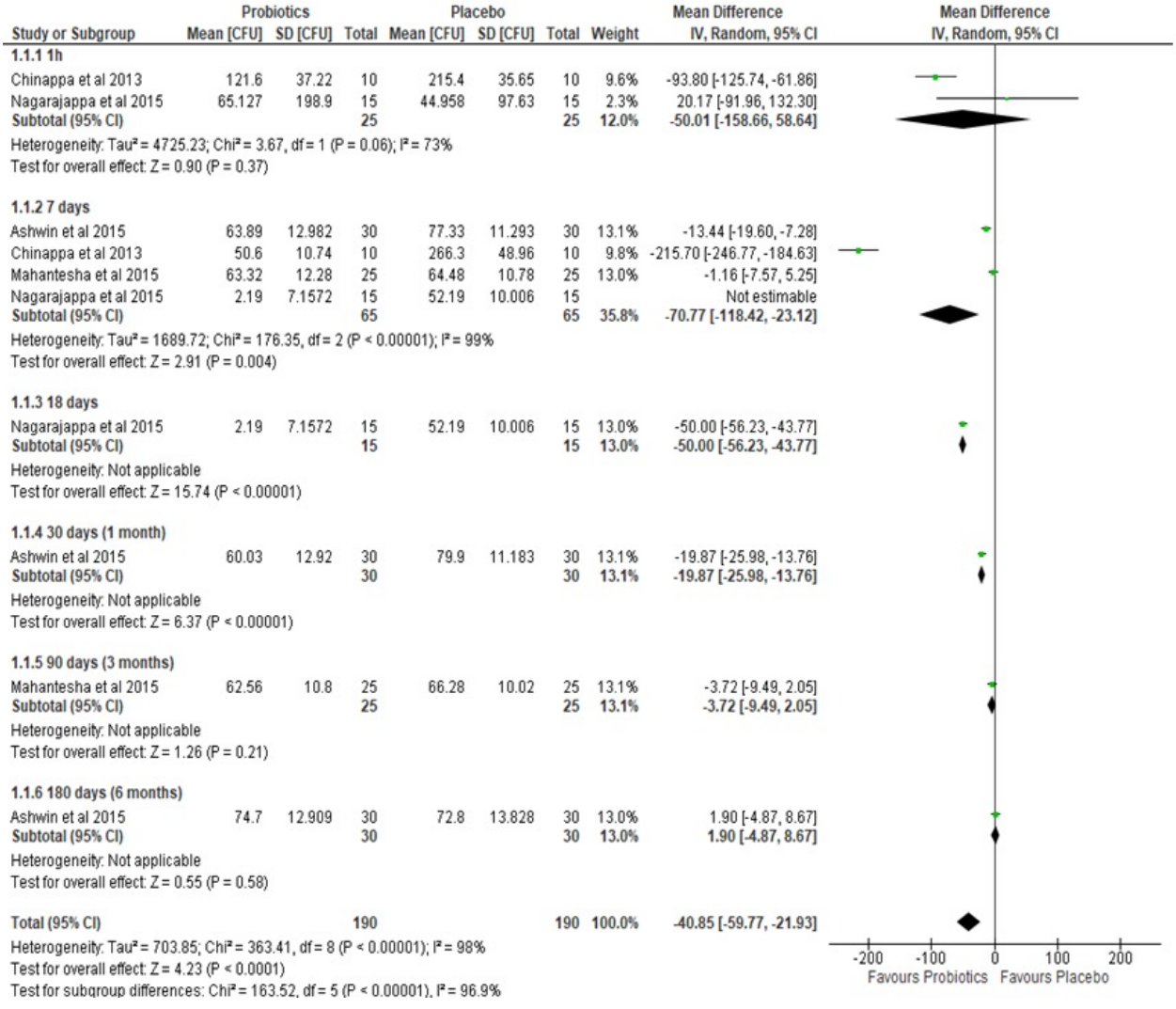

Figure 2. Forest plot of comparison: Ice-cream containing probiotics versus ice-cream without probiotics or drink with probiotics.

DISCUSSION

The strategic incorporation of probiotics in dental caries management epitomizes a novel paradigm in contemporary oral health research. This systematic review meticulously scrutinizes the efficacy of probiotic-fortified ice cream in attenuating the salivary concentrations of *Streptococcus mutans*. Notably, this study reveals a substantive decrement in *S. mutans* load, sustained for up to 7 days subsequent to the ingestion of probiotic-infused ice cream.

The realm of functional foods, particularly dairy-based products, has been recognized as a conducive matrix for probiotic delivery (10, 11, 27). Expanding upon this notion, this study delineates the potential role of ice cream as a palatable and efficacious probiotic carrier. This is particularly relevant in pediatric contexts, where acceptability and compliance with health interventions are paramount. The comparative analysis undertaken by Mahantesha *et al*. (2015) (24) accentuates the superiority of ice cream, relative to other probiotic vehicles, in mitigating *S. mutans* levels.

Delving deeper, the research by Çaglar *et al*. (2008) (21) delineates a significant reduction in *S. mutans* in young adults following a regimen of B. lactis Bb-12 enriched ice cream. This not only illuminates the strain-specific efficacy in oral health interventions but also highlights the nonexistence of detrimental effects. The researchers, however, astutely acknowledge the constraints of their study, including limited sample size and the transient nature of salivary mutans streptococci as an intermediate marker for caries progression.

In a similar vein, the incorporation of bifidobacteria in a chocolate-based ice cream was observed to reduce salivary *S. mutans* and *lactobacilli* by 26.8% over an 18-day period in adult participants (25). The intrinsic attributes of ice cream, enriched with casein, calcium, and phosphorus, coupled with its distinctive textural and sensory characteristics, amplify its suitability as a probiotic conduit (9).

Moreover, this review elucidates a consistent reduction in *S. mutans* across diverse pediatric studies, with the duration of probiotic consumption emerging as a critical determinant in the efficacy of these interventions (22-26). The probiotic-induced biofilm formation acts as a defensive barrier, mitigating cariogenic microorganisms and suggesting a viable preventive approach in pediatric dentistry (11,28).

Diverse concentrations of *B. lactis* Bb-12 and L. acidophilus La-5 were employed across the studies under review (21-26), each strain exhibiting unique properties conducive to oral health. *B. lactis* Bb-12 is renowned for its adhesion capabilities, crucial for effective colonization in the oral cavity (29), while *L. acidophilus* La-5 is distinguished by its bacteriocin production, instrumental in targeting *S. mutans* within the oral biofilm (30, 31). Yet, the heterogeneity in administration protocols underscores the necessity for standardized methodologies in future research endeavors.

A critical limitation of this review is the exclusion of a sensitivity analysis to appraise the heterogeneity across the included studies, a facet that could potentially influence the interpretative breadth of our findings (32). The variations in enrichment protocols and follow-up durations further necessitate a nuanced interpretation of the results. Despite these challenges, this review represents an inaugural comprehensive synthesis of randomized controlled trials exploring the efficacy of probiotic-enriched ice cream in diminishing *S. mutans* counts, a pivotal step in dental caries prevention.

It is imperative to underscore that while certain probiotics like B. lactis Bb-12 exhibit resilience in low pH environments, indicative of their potential

efficacy in the oral milieu (29), their acidogenic propensity in symbiosis with *S. mutans* raises concerns regarding enamel demineralization in complex biofilm matrices (16). Consequently, future investigations must be directed towards unraveling the in situ interactions between probiotics and oral biofilms, identifying optimal probiotic strains and dosages, and elucidating their long-term implications on oral health, particularly concerning enamel integrity and the demineralization-remineralization dynamic (33).

CONCLUSION

Probiotic-enriched ice cream, particularly containing strains such as *B. lactis* Bb-12 and *L. acidophilus* La-5, significantly reduces salivary levels of *Streptococcus mutans*. The uniformity in the reduction of *S. mutans* across different study populations underlines the potential of probiotic ice cream as a universally acceptable and effective medium.

AUTHOR CONTRIBUTION STATEMENT

Conception or design: P.H.S.S., R.S.A., R.O.C., S.I.C.S., L.R.A.L. and M.J.F.C.
Acquisition, analysis, or interpretation of data: P.H.S.S., R.S.A., R.O.C., S.I.C.S., L.R.A.L. and M.J.F.C.
Drafting the work or reviewing it critically for important intellectual content: S.I.C.S., L.R.A.L. and M.J.F.C.
Final approval of the version to be published: P.H.S.S. and M.J.F.C.

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