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REVIEW ARTICLE

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The effects of nitrate on the oral microbiome: a systematic review investigating prebiotic potential

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ABSTRACT

Background: Nitrate (NO_3^-) has been suggested as a prebiotic for oral health. Evidence indicates dietary nitrate and nitrate supplements can increase the proportion of bacterial genera associated with positive oral health whilst reducing bacteria implicated in oral disease(s). In contrast, chlorhexidine-containing mouthwashes, which are commonly used to treat oral infections, promote dysbiosis of the natural microflora and may induce antimicrobial resistance.

Methods: A systematic review of the literature was undertaken, surrounding the effects of nitrate on the oral microbiota.

Results: Overall, $n = 12$ *in vivo* and *in vitro* studies found acute and chronic nitrate exposure increased (representatives of) health-associated *Neisseria* and *Rothia* (67% and 58% of studies, respectively) whilst reducing periodontal disease-associated *Prevotella* (33%). Additionally, caries-associated *Veillonella* and *Streptococcus* decreased (25% for both genera). Nitrate also altered oral microbiome metabolism, causing an increase in pH levels ($n = 5$), which is beneficial to limit caries development. Secondary findings highlighted the benefits of nitrate for systemic health ($n = 5$).

Conclusions: More clinical trials are required to confirm the impact of nitrate on oral communities. However, these findings support the hypothesis that nitrate could be used as an oral health prebiotic. Future studies should investigate whether chlorhexidine-containing mouthwashes could be replaced or complemented by a nitrate-rich diet or nitrate supplementation.

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Nitrate; nitrite; nitric oxide; periodontal disease; chlorhexidine; antimicrobial resistance

Introduction

Dietary NO_3^- is a polyatomic ion that naturally occurs in vegetables, particularly leafy greens (lettuce, kale and spinach) and beetroots [1–6]. Additionally, NO_3^- is commonly added to processed meats for flavouring, preservation and antimicrobial purposes [2]. Evidence suggests NO_3^- added to processed meats can lead to the formation of carcinogenic nitrosamines [4]. However, ≈ 80% of dietary nitrate is obtained from vegetables which are considered anticarcinogenic [4,7]. Moreover, plant-based NO_3^- exerts positive effects on oral and systemic health [1,2,4]. NO_3^- consumption leads to the production of nitric oxide (NO), a bioactive molecule associated with host defence, neuronal communication, improved vascular and metabolic health, and improved exercise performance [1,4–7]. Importantly, NO is a potent vasodilator that regulates blood pressure (BP) and blood flow within tissues and organs. High NO_3^- diets are associated with a reduced risk of cardiovascular disease (CVD), diabetes and cognitive impairment, as increased NO bioavailability enhances the delivery of oxygen and nutrients to body systems [4,6]. Interestingly, NO can also be produced endogenously from L-arginine and NADPH in an oxygen-dependent reaction involving NO synthase enzymes (NOS), of which there are three main types (endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS)) [8]. Unfortunately, endogenous NO production becomes less efficient with age [3–5]. However, dietary NO_3^- is reduced to NO via the nitrate-nitrite-nitric oxide pathway (NO_3^--NO_2^--NO) (Figure 1) [1,3,4,6]. This process is facilitated by nitrate-reducing bacteria (NRB) inside the oral cavity, including *Rothia* and *Neisseria*, which are associated with positive oral health [7,8]. High-nitrate diets have been shown to increase health-associated genera and decrease the number of disease-associated bacteria [7,8].

In contrast, some bacteria are linked to the development of dental caries (e.g. *Lactobacillus* and cariogenic representatives of *Streptococcus, Veillonella* and *Actinomyces*), whilst others are linked to the development of halitosis and periodontitis (e.g. *Porphyromonas, Fusobacterium* and *Prevotella*) [7,8]. Cariogenic bacteria ferment carbohydrates (e.g. glucose and sucrose) into organic acids (e.g. lactic acid) which

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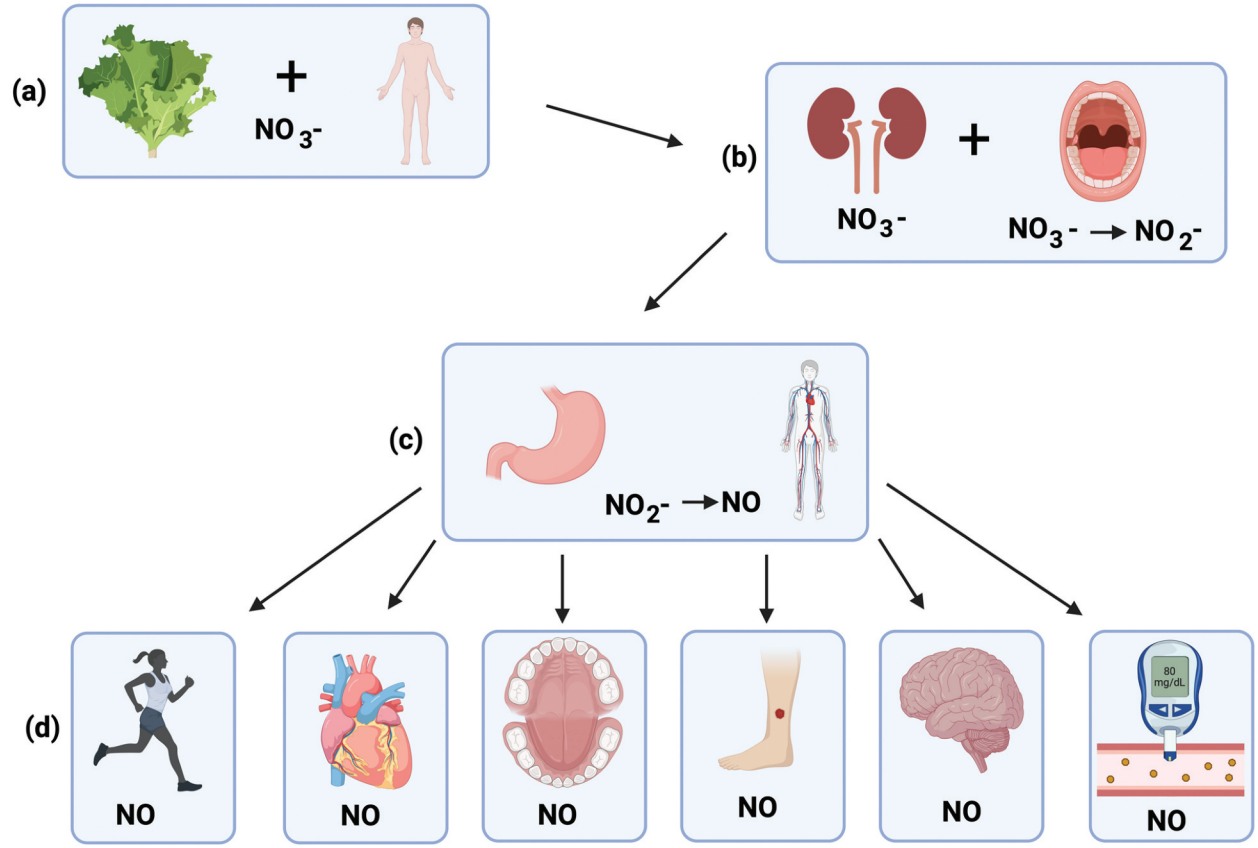

Figure 1. Schematic representation of the NO_3^--NO_2^--NO pathway (a) Nitrate-rich vegetables are consumed, NO_3^- enters the GI tract and is absorbed into the bloodstream (b) about 75% of NO_3^- is excreted in urine, whilst approximately 25% is concentrated by the salivary glands (facilitated by the transporter protein sialin) and is reduced to NO_2^- by bacteria inside the oral cavity (c) NO_2^- is then swallowed and reduced to NO in the stomach and/or other body tissues after entering the systemic circulation (d) NO induces health benefits, including improvements to exercise performance, cardiovascular health, oral health, wound healing, cognitive function and blood glucose levels (created with BioRender.com).

can decrease oral pH and cause demineralisation of tooth enamel [6,7]. Studies have shown NO_3^- limits oral acidification via lactic acid/proton consumption during denitrification and nitrite reduction to ammonium by oral bacteria, which can prevent caries development [4,6–8]. Additionally, periodontal disease (PD) is a chronic oral infection that affects 20–50% of the global population [9–11]. PD is characterised by chronic inflammation, bleeding gums and destruction of the periodontium; the supporting tissue of the teeth [11]. Although the condition is multifactorial, complex biofilms (dental plaque) containing a large diversity of bacterial species contribute to PD manifestation and progression [7,9,10]. Interestingly, some studies have found PD patients have high concentrations of NO in their saliva and gingival crevicular fluid, potentially due to excessive iNOS activation driven by proinflammatory cytokines which can contribute to tissue damage [8,11]. In contrast, dietary NO_3^- appears to induce a controlled, low-level production of NO that is beneficial for oral health [7,8].

Treatment for oral disease(s) typically involves mechanical plaque removal and the use of chlorhexidine-containing mouthwashes (CHX) to reduce gingival inflammation [9,10]. Unfortunately, prolonged use of CHX mouthwash can disrupts the oral microbiota, displacing NO_3^- reducing species and lowering oral pH, in addition to negatively affecting the expected BP reduction following an NO_3^- dose [4,8]. Moreover, evidence suggests that antimicrobial resistance (AMR) is developing against antibacterial mouthwash, thus promoting cross-resistance to antibiotics [4,10,12]. Worryingly, annual deaths related to AMR are expected to reach 10 million yearly by 2050 [13]. Thus, the emergence of resistant strains can induce a life-threatening infection inside a compromised host and/or spread environmentally [10,13,14]. In terms of periodontitis, CHX resistance would enable disease-causing bacteria to increase and exacerbate oral disease progression [7,10].

Several studies have suggested that dietary NO_3^- should be investigated as a prebiotic for oral health, as a mechanism to prevent or treat oral disease(s) [4,7,15–18]. Prebiotics are food components that promote the growth of beneficial microorganisms [7]. Using NO_3^- as a prebiotic could reduce the over-reliance on CHX mouthwashes by giving health-associated species a growth advantage and potentially

limiting the growth of disease-associated species [4,7]. To address this hypothesis, a systematic review of the literature was performed with the scope to answer the following research question: *What are the effects of nitrate on the oral microbiota?*

Methodology

Overview

Evidence presented in this review to satisfy the research aim was derived from clinical and *in vitro* studies. A narrative synthesis will highlight the effects of NO_3^- on the oral microbiome.

Search strategy

This review was conducted following PRISMA (2020) guidelines [19]. Four databases were accessed (Table 1). All relevant papers came from peer-reviewed journal articles. The search criteria narrowly focused on the effects of NO_3^- on oral microorganisms. A date restriction was applied (2012–2022) to find the most up to date information relevant to the topic. Key words combined with Boolean operators and search strings were used to confine search results to the topic of interest (Table 1). Mendeley and Microsoft Excel were used for record keeping. A PRISMA diagram is shown in the Results section.

Inclusion and exclusion criteria

Material to be included in the review: 1) Peer-reviewed *in vitro* and *in vivo* studies analysing the effect of NO_3^- on oral community composition and activity 2) Studies published between 2012–2022 3) no restrictions for country, participant gender, age and race.

Material to be excluded from the review: 1) Studies that did not analyse complex oral microbiota samples (saliva, tongue samples or dental plaque) 2) duplicate research papers 3) non-relevant articles and/or articles containing insufficient information to answer the research question 4) non-English language articles 5) book chapters, conference notes, reviews and theses 6) articles with no full-text access.

Quality assessment checks

Relevant studies were analysed against a Scottish Intercollegiate Guidelines Network flowchart (2022) to determine study type [20,21] (Appendix A). Studies were established to be randomised control trials (RCTs), Quasi-experiments (non-randomised control studies (non-RCS) and pre-post interventions), cross-sectional studies, and *in vitro* analyses. All studies were quality assessed against critical appraisal tools to determine validity and suitability.

Each RCT was quality assessed against two critical appraisal tools provided by the Center for evidence-Based Management and Critical Appraisal Skills Programme [22,23]. Quasi-experimental studies were critically appraised against tools provided by the British Medical Journal and The Joanna Briggs Institute [24,25]. Similarly, cross sectional studies were quality assessed against two appraisal tools provided by the Center for evidence-Based Management and STROBE [26,27]. Lastly, *in vitro* studies were critically appraised using a checklist provided by the University of Exeter and The QUIN tool [28,29] (See appendix B-I). All tools had a checklist format and contained key questions highlighted in Table 2.

Most tools consisted of optional answers, including 'yes', 'no', 'unclear' or 'can't tell". Good quality studies should return a majority of 'yes' answers. If

Table 1. Search strategy used during systematic review.

Main Concept	Keywords/key terms	Boolean Operators	Databases
Search Strategy			
The effects of nitrate on oral microbiome composition and activity	nitrate effects impact "oral microbiome" "oral microbiota"	AND OR	Scopus NCBI Science Direct Google Scholar
Search string 1: (effects OR impact) AND (nitrate) AND ("oral microbiome" OR "oral microbiota")			

Table 2. Common quality assessment questions for RCTs, quasi-experiments, cross-sectional studies and *in vitro* analyses.

Typical critical appraisal questions
Did the study have a focused research question?
Was the study design valid?
Was the sample size adequate and target population representative?
Was there a clear method of selection/randomisation and/or a control group?
Was the intervention/exposure clearly reported?
Were outcomes measured reliably and are results clear and unbiased?
Have confounders been identified?
Will the results help locally/target populations?

appraisal results caused uncertainty regarding inclusion or exclusion, the project team were consulted for a second opinion. Overall, selected papers had a good critical appraisal result.

Data collection and narrative synthesis

A summary table was created in Microsoft Word to store key information from each study (Table 3). Key trends, similarities, and differences between studies were explored and an overall conclusion relating to the research question was reached.

Results

Prisma diagram and summary table

Applying the search strategy outlined in Section 2.3 returned 3133 potential articles (Figure 2). This total was reduced to 1943 after removing 1190 duplicates. Thereafter, title and abstract screening removed a further 1745 non-related articles, leaving 198 articles to be fully screened. Subsequently, a further 188 papers met the exclusion criteria (section 2.4). Thus, 10 articles satisfied the inclusion guidelines and featured in the review. Additionally, 2 further papers were found through screening the reference lists of eligible articles. Overall, 12 studies satisfied the inclusion criteria (Table 3).

Study characteristics

Several study types were identified from this systematic approach: 7 were randomised control trials (RCTs), 3 were *in vitro* analyses and 2 demonstrated a Quasi experimental design (Table 4). Moreover, studies were highly variable by location. Additionally, 11 of the studies reported that NO_3^- induced oral microbiome changes, with 8 emphasising NO_3^- had a positive impact on oral health. Lastly, several studies (n=6) reported NO_3^--associated systemic benefits, predominantly improvements to cardiovascular health.

Laboratory investigations

The most common laboratory procedures for NO_3^-/NO_2^- quantification included chemiluminescence, chromatography, the Griess colorimetric method and the RQflex® reflectometer. Additionally, 16s rRNA gene Illumnia Sequencing was predominantly used for bacteria identification/quantification. Typically, the V3-V4 region of the 16s rRNA gene was amplified, using, the 341F and 806R or 805R primer set (Table 5).

Bacteria constituents

Several bacterial genera were identified across all studies, including gram-positive and gram-negative genera, with and without nitrate producing capacity (NPC). Moreover, some genera contained spp. that are associated with oral disease and dental caries (Table 6).

Nitrate supplementation induced modifications in oral communities

Post NO_3^- supplementation, there is an increase in bacterial spp. associated with good oral health, whilst a decrease is observed in genera linked to oral disease (Table 7). For instance, *Neisseria* and *Rothia* increased the most, at 67% and 58%, respectively (Table 6). In terms of percentage decreases, *Prevotella* and *Veillonella* decreased the most across all studies, at 33% and 25%, respectively.

Additionally, eleven studies reported an increase in NRB associated with eubiosis, whilst five studies reported a decrease in bacterial species associated dysbiosis (Table 8). Interestingly, five studies reported both an increase in NRB associated with eubiosis and a decrease in bacterial genera associated with dysbiosis. Lastly, five studies reported oral pH changes post NO_3^- treatment.

Additional findings

Five studies reported other *in vivo* health benefits, including improvements to cardiovascular and cognitive health (Table 9). Moreover, one study emphasised that NO_3^- supplementation could be used for plaque control or to treat periodontitis. Lastly, a study discussed the detrimental impact of antiseptic mouthwash on oral communities (Table 9).

Discussion

NO_3^- promotes eubiosis inside the oral cavity

NO_3^- supplementation increased oral health-associated NRB, particularly *Rothia* and *Neisseria*, whilst reducing generally disease-associated *Prevotella, Veillonella* and *Streptococcus* (Table 7). Collectively, most studies (n=11) reported an increase in NRB associated with eubiosis and/or a decrease in bacterial spp. associated with dysbiosis, post NO_3^- supplementation (Table 8). Eubiosis refers to a balanced symbiotic relationship between oral microbes [40–42]. In contrast, dysbiosis occurs when insults to oral ecology, such as sugar consumption, smoking, poor dental hygiene or antimicrobial mouthwash, disrupt this commensal relationship, which causes disease-associated bacteria to increase [41–46]. In PD, dysbiosis activates an excessive

Table 3. Studies assessing NO_3^- supplementation and oral ecology (2012–2022).

First author, Year and journal	Study Type and Location	Aim/participant information	Laboratory investigation(s)	Results/Findings	Author's Conclusions
(1) Velmurugan *et al.*, 2016 The American Journal of Clinical Nutrition [30]	*In vivo*: randomised, double-blind, placebo-controlled parallel study (UK)	The effects of dietary NO_3^- on vascular and platelet function, and the oral microbiome n = 67 hypercholesterolemic M and F participants Age: 18–80 yrs old BMI: 18.5–40 kg/m^2	BR juice vs PL (6-wk, once-daily) **Analyses**: Vascular and platelet function, BP, saliva, urine and plasma NO_3^-/NO_2^- levels and oral microbiome (saliva) **Methods**: FMD, aPWV, portable BP device, flow cytometry, chemiluminescence and16s rRNA gene Illumina Sequencing	In the treatment group: FMD and aPWV improved ($p < 0.001$ and $p = 0.06$, respectively); platelet monocyte aggregates significantly reduced ($p < 0.004$); *Rothia mucilaginosa* increased and *Neisseria flavescens* significantly increased ($p < 0.01$)	Chronic dietary NO_3^- consumption improves vascular function in hypercholesterolemic individuals; changes are related to alterations in the oral microbiome/increased levels of NRB
(2) Koopman *et al.*, 2016 Microbial Ecology [31]	*In vitro* study (Netherlands)	Assessing the effects of dietary NO_3^- on oral ecology and biochemistry MAM biofilm model inoculated with stimulated saliva from a healthy M and F donor Age: 20–30 yrs old	n=4 microcosms received NO_3^-, n = 4 microcosms acted as control; all received weekly NO_3^- dose and sucrose pulse (4-wk exp) **Analyses**: NO_3^-, and NO_2^- concentrations, oral pH, short chain fatty acid production and bacterial composition **Methods**: Capillary electrophoresis, pH meter and 16s rRNA gene Illumina Sequencing	In one donor, NO_3^- supplementation significantly increased *Neisseria* on a genus-level. In both donors, *Neisseria* OTUs were associated with NO_3^- supplementation and in one donor, *Veillonella* OTUs. Butyrate levels were lower after nitrate addition in NO_3^- receiving microcosms ($p < 0.05$)	NO_3^- affects the composition/ biochemistry of oral microbiomes, but changes vary depending on the inoculum (donor)
(3) Vanhatalo *et al.*, 2018 Free Radical Biology and Medicine [32]	*In vivo*: randomised, double-blind, cross-over trial (UK)	Examining relationships between oral ecology and physiological indices of NO bioavailability, and assessing the impact of NO_3^- supplementation on these variables n = 18 normotensive M and F Age: 19–22 yrs old; 70–79 yrs old	BR juice vs PL (10 days, twice-daily) **Analyses**: oral ecology (tongue and saliva), plasma, NO_3^- and NO_2^- concentrations, BP and arterial stiffness **Methods**: 16s rRNA gene Illumina Sequencing, chemiluminescence, automated sphygmomanometer MAP measurements and PWV	NO_3^- supplementation increased NO_3^-/NO_2^- plasma concentrations ($p < 0.05$); *Rothia/Neisseria* genera increased and *Prevotella/Veillonella* decreased vs PL ($p < 0.05$). SBP and MAP were significantly lower in the older age group vs PL ($p < 0.05$)	Dietary NO_3^- altered the oral microbiota and changes in oral ecology positively impacted NO homeostasis and vascular health
(4) Ashworth *et al.*, 2019 Free Radical Biology and Medicine [33]	*In vivo: n*on-randomised, single blinded, cross-over trial (UK)	Evaluating dietary NO_3^- intake in vegetarians vs omnivores and the impact of AM on NO_3^- NO_2 levels, RMR, BP and the oral microbiome. Healthy M and F participants n = 22 vegetarians n = 19 omnivores Age: 18–45 yrs old	PL vs Corsodyl (2-wk trial) **Analyses**: RMR, BP, plasma and salivary NO_3/NO_2^- concentrations, saliva pH, blood glucose and lactate levels, and oral ecology (saliva) **Methods**: RMR calculations, oscillometric device, chemiluminescence, biochemistry analyser, digital pH meter and 16s rRNA gene Illumina Sequencing	No significant differences in NO_3^-/NO_2^- concentrations, NRC, and oral ecology between groups following PL ($p > 0.05$). AM decreased NO_2^- levels ($p<0.05$), NRC and oral pH, and increased salivary lactate and glucose levels in both groups (all $p < 0.001$) (*Prevotella*, *Actinomyces*, *Rothia* and *Fusobacterium* genera decreased ($p < 0.05$))	Dietary NO_3^- consumption in vegetarians vs omnivores was not statistically different.

(*Continued*)

Table 3. (Continued).

First author, Year and journal	Study Type and Location	Aim/participant information	Laboratory investigation(s)	Results/Findings	Author's Conclusions
(5) Burleigh *et al.*, 2019 Nitric Oxide [15]	*In vivo* placebo-controlled, single-blind randomised cross over trial (UK)	Analysing the effects of dietary NO_3^- on oral microbiome composition and NRC, and assessing vascular responses to acute NO_3^- ingestion. n = 11 healthy M Age: 30 ± 7 yrs old BM: 86.9 ± 14.1kg	BR juice vs PL (7 days, twice daily) **Analyses**: plasma and salivary NO_3^-/NO_2^- concentrations, microbiome (tongue), oral pH, HR, BP and endothelial functioning **Methods**: chemiluminescence, 16s rRNA gene Illumina Sequencing, pH-meter, FMD, automated BP monitor, telemetry and MAP measurements	Plasma/saliva NO_3^- and NO_2^- levels and salivary pH increased post NO_3^- supplementation (p<0.05). *Prevotella, Actinomyces* and *Streptococcus* genera decreased in the BR group; *Neisseria subflava* increased in both groups compared to baseline, but this increase was most significant for the BR group compared to PL (p = 0.001)	Dietary NO_3^- altered the oral microbiome in favour of oral health, but changes did not enhance NRC or vascular responses to acute NO_3^- ingestion
(6) Liddle *et al.*, 2019 Nitric Oxide [34]	*In vivo: b*efore-and-after study (UK)	Analysing CV_B, CV_A and CD for plasma and saliva NO_3^-/NO_2^- concentrations pre and post-ingestion of BR juice, and assessing the CV_B of NR bacteria on the tongue n = 10 Recreationally active M Age: 25 ± 5 yrs old BM: 81 ± 11 kg	BR juice (3 identical trials, 6–10 days apart) **Analyses**: salivary, plasma and urine NO_2^- and NO_3^- concentrations, BP, MAP, microbiome (tongue) **Methods**: chemiluminescence, oscillometric device, MAP measurements and16s rRNA gene Illumina Sequencing	Profound CV_B variations in saliva and plasma NO markers, and NR bacteria at baseline vs post BR ingestion. *Neisseria subflava* was negatively associated with peak saliva/plasma NO_2^- levels; *Rothia mucilaginosa* and *Haemophilus parainfluenzae* were positively and negatively associated with between-trial peak saliva/plasma NO_2^- levels (all $p < 0.05$). BR ingestion reduced BP	There is profound CV_B in oral NR bacterial abundance on the tongue and NO metabolites in plasma/saliva pre and post-ingestion of BR
(7) Rosier *et al.*, 2020 Scientific Reports [7]	*In vitro* study (Spain)	Establishing the short-term effect of a growth medium containing NO_3^- dose on oral biofilm growth, composition and biochemistry n = 12 saliva samples from healthy donors	Samples were incubated in nutrient-rich media with or without NO_3^- and examined at 5h and 9h **Analyses**: NO_3^-/NO_2^- levels, ammonium, lactate and pH measurements, protein and DNA quantification **Methods**: Reflectoquant reflectometer, Bradford protein assay and 16s rRNA gene Illumina Sequencing	NO_3^- increased ammonium and pH levels, and decreased lactate production (all p<0.01) NO_3^- increased abundance of *Neisseria* and *Rothia* genera after 5h (p<0.01) and reduced *Streptococcus, Oribacterium, Veillonella, Fusobacterium, Leptotrichia, Prevotella, Alloprevotella* and *Porphyromonas* after 5 and/or 9h (p<0.05)	NO_3^- significantly altered the oral environment to benefit the host and could serve as a prebiotic for oral health
(8) Rosier *et al.*, 2020 Frontiers in Microbiology [35]	*In vitro* study (Spain)	Isolating oral NR bacteria and assessing their *in vitro* probiotic capabilities **Probiotic isolation**: n = 5 healthy M and F donors Age: 23–32 yrs old **Saliva donors**: n = 9 healthy M and F donors Age: 23–45 yrs old	6 *Rothia* sp. underwent *in vitro* biofilm tests (saliva biofilms). **Analyses**: NRC, bacterial identification; NO_3^-/NO_2^- levels, ammonium, lactate and pH measurements **Methods**: cell culture, Griess reaction, 16s rRNA gene Illumina Sequencing, Reflectoquant reflectometer and the Nessler method	Biofilm NRC increased when adding isolates compared to controls (p<0.05). Adding NO_3^- or NO_3^- + isolates caused a smaller decrease in pH (p<0.05) and some isolates in combination with NO_3^-increased lactate consumption. A positive correlation between NRC and ammonium production was observed, even in samples without NO_3^- (p<0.01)	Oral communities with reduced NRC could benefit from a NR probiotic or symbiotic combination (NO_3^- and NR probiotic)

(*Continued*)

Table 3. (Continued).

First author, Year and journal	Study Type and Location	Aim/participant information	Laboratory investigation(s)	Results/Findings	Author's Conclusions
(9) Vanhatalo *et al.*, 2021 Redox Biology [36]	*In vivo*: randomised, placebo-controlled cross-over trial (UK)	Assessing relationships between cognitive and physiological traits and clusters of co-occurring oral bacteria *n* = 30 elderly M and F Age: 70–80 yrs old	BR juice vs PL (10 day trial) **Analyses**: plasma NO_3^-/NO_2^- levels, microbiome (saliva), cognitive function, BP, arterial stiffness, exercise assessments, muscle function, brain matter **Methods**: chemiluminescence, 16s rRNA gene Illumina Sequencing, WGCNA, cognitive function tests, BP measurement, PWV, MAP measurements, VO_2 max, p-MRS of quadriceps and H-MRS of brain	*Rothia-Streptococcus* microbiome module increased after NO_3^- supplementation and showed stable associations with cardiovascular indices of health; *Neisseria-Haemophilus* module increased and showed stable associations with cognitive indices of health. Co-occurring *Prevotella-Veillonella* diminished after NO_3^- ingestion	NO_3^- sensitive oral microbiome modules could be potential pre- and probiotic targets to modify age-associated cardiovascular and cognitive impairments
(10) Sinha *et al.*, 2021 Environmental Research [37]	*In vivo*: randomised control trial (USA)	Analysing the effects of NO_3^-/NO_2^- processed meat and nitrate-enriched drinking water on salivary microbial populations *n* = 63 healthy M and F volunteers Age: 18–70 yrs old BMI: 18–25 kg/m^2	2 groups: 3 meat intervention phases and 1 meat intervention phase + nitrate-enriched water (7-week trial) **Analyses**: urinary NO_3^-/creatinine levels, microbiome (saliva) **Methods**: ion chromatography and mass spectrometry, Beckman clinical analyser (colorimetric method) and 16s rRNA gene Illumina Sequencing	Urinary NO_3^- levels were reduced during the phytochemical-enriched-low- NO_2^- meat diet and poultry diet compared to baseline ($p < 0.009$). NO_3^- rich water induced changes to salivary microbiome ($p < 0.001$). *Neisseria, Kingella, Lautropia, Alloprevotella* and *Haemophilus* genera increased; *Streptococcus* decreased (FDR < 0.05)	Drinking NO_3^- enriched water increases oral NR bacteria abundance, which can subsequently increase NOC production
(11) Rosier *et al.*, 2021 Frontiers in Cellular and Infection Microbiology [38]	*In vivo*: placebo-controlled, blinded, cross-over trial (study 1) and before-and-after study (study 2 & 3) (Spain)	Evaluating the ability of NO_3^- to reduce oral acidification caused by sugar fermentation *in vivo* **Study 1**: *n* = 12 healthy M and F participants, age: 25–60 yrs old **Study 2**: *n* = 6 healthy M and F participants, age:24–46 yrs old **Study 3**: *n* = 6 healthy M and F participants, age: 25–33 yrs old	Sucrose rinses 1, 2 and 4h after NO_3^- ingestion (BR vs placebo) **Analyses**: salivary NO_3^-/NO_2^- concentrations, microbiome (saliva), pH levels, lactate and ammonium measurements **Methods**: Reflectoquant reflectometer, 16s rRNA gene Illumina Sequencing, the Nessler method and lactate colorimetric assay	**Sucrose rinse 4hrs**: NO_3^- supplementation reduced lactate production ($p < 0.05$), increased pH and *Rothia* genera abundance ($p < 0.05$). Relative abundances of *Neisseria* and *Rothia* negatively correlated with lactate production, and *Neisseria* abundance positively correlated with pH post sucrose intake ($p < 0.05$)	NO_3^- can limit oral acidification induced by sugar fermentation, predominantly due to lactate usage by NR bacteria
(12) Jockel-Schneider *et al.*, 2021 Journal of Periodontology [17] (continuation Jockel-Schneider *et al.*, 2016 [16])	*In vivo*: randomised control trial (Germany)	Evaluating the impact of a NO_3^--rich diet on NO_3^- fermenting bacterial abundance within the pocket microbiota *n* = 37 periodontal recall patients with gingival inflammation and reduced periodontium	lettuce juice vs PL (14 days, three times daily) **Analyses**: subgingival microbial samples (pre and post plaque removal) and salivary NO_3^-/NO_2^- levels **Methods**: anion exchange chromatography and16s rRNA gene Illumnia Sequencing	No baseline differences in bacterial diversity between the BR and PL group ($p > 0.05$) NO_3^- supplementation induced significant increases in *Rothia* and *Neisseria* genera ($p < 0.05$) and significantly altered alpha- and beta- diversity ($p < 0.05$) compared to PL group	NO_3^- supplementation in periodontal recall patients reduced gingival inflammation and induced significant changes to subgingival microbial populations; NO_3^- could be used as an adjunct therapy to plaque removal to control gingival inflammation

M = male, F = female, BMI = body mass index, BR = beetroot, PL = placebo, wk = week, FMD = flow-mediated dilation, aPWV = aortic pulse wave velocity, BP=blood pressure, rRNA = ribosomal ribonucleic acid, NRB = nitrate reducing bacteria, MAM = multiplaque artificial mouth, exp = experiment, MAP = mean arterial pressure, PWV = pulse wave velocity, SBP = systolic blood pressure, AM = antibacterial mouthwash, RMR = resting metabolic rate, NRC = nitrate reducing capacity, HR = heart rate, CVB= within-person biological variation, CVA = analytical imprecision, CD = critical difference, NR = nitrate reducing, WGCNA = weighted gene co-expression network analysis, VO2 max = maximal oxygen consumption, p-MRS = phosphorus magnetic resonance spectroscopy, H-MRS = proton magnetic resonance spectroscopy, NOC = N-nitroso compounds, OTU = operational taxonomic unit.

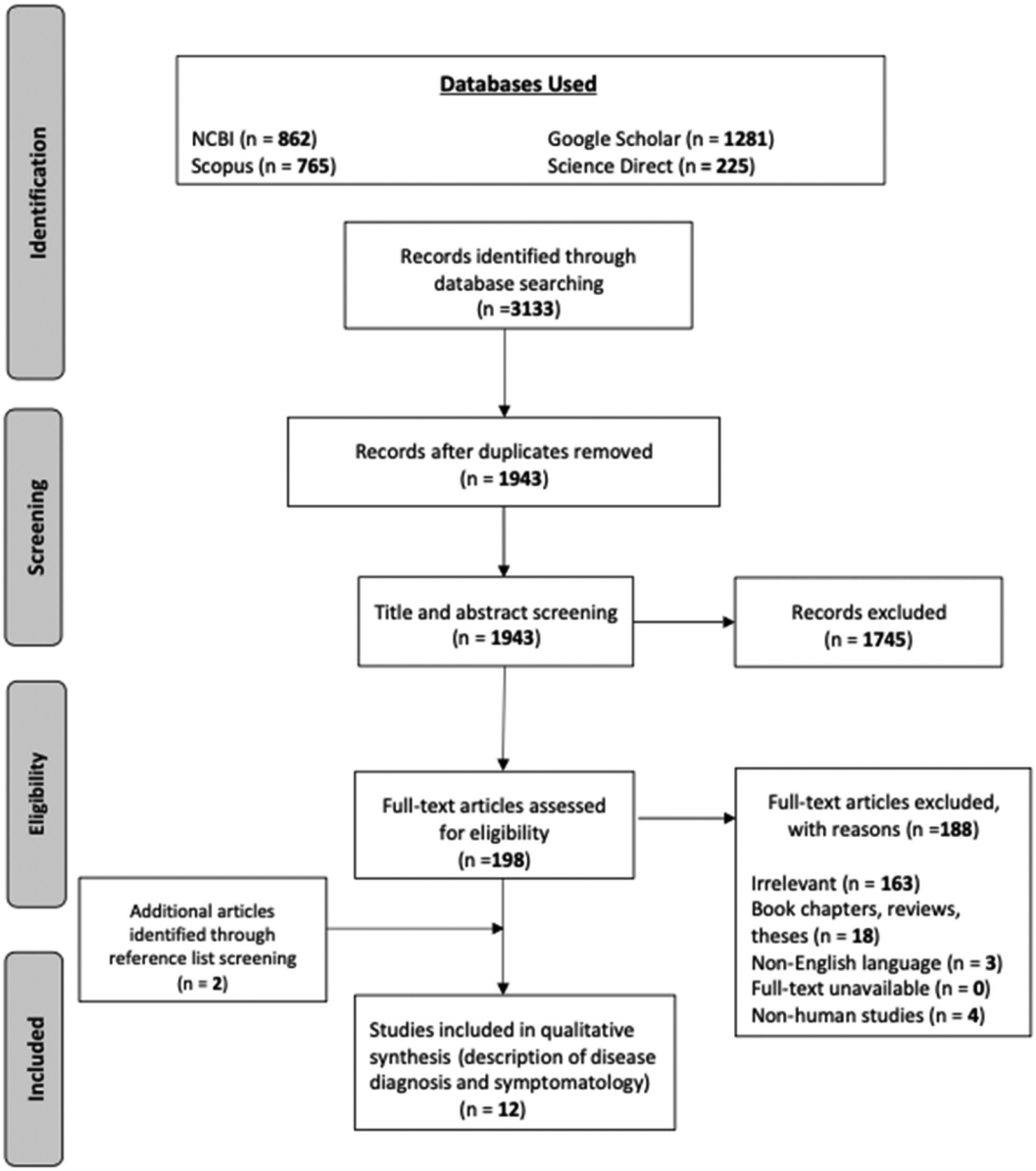

Figure 2. PRISMA flow diagram.

Table 4. Summary of study characteristic (n = 12)

Study type	
RCTs	7
in vitro	3
Quasi exp (non-RCTs and pre-post interventions)	2
Location	
UK	6
Spain	3
USA	1
Netherlands	1
Germany	1
NO_3^- altered the oral microbiome	
Yes	11
impact on oral health	
Positive	8
*Unspecified	4
NO_3^- and other health benefits	
CV health	5
Cognitive health	1

*studies were investigating the impact of NO_3^- induced oral microbiome changes on other parameters (e.g. cardiovascular health), and did not directly analyse the positive or negative impact of NO_3^- on oral health.

inflammatory response that destroys host tissues, while in dental caries, dysbiosis can contribute to acidification and enamel demineralisation [11,41]. Overall, NO_2^- and NO production via the NO_3^--NO_2^--NO pathway promotes microbial homeostasis by exerting antibacterial effects against certain disease-associated bacteria, potentially maintaining a balanced microenvironment that lowers oral disease risk [8]. Thus, findings from this review indicate NO_3^- can alter the oral microbiota in favour of oral health, highlighting that it may have a protective role against the development of oral disease, or the potential to be used as a therapeutic intervention.

Additionally, studies that measured oral pH (n=5) found acute and chronic NO_3^- consumption beneficially altered oral biochemistry, by increasing salivary

Table 5. Most common laboratory procedures across all studies.

NO_3^- and NO_2^- Quantification	Bacterial ID/quantification (primers)
Chemiluminescence, The Griess colorimetric method, chromatography and Reflectoquant RQflex®	16s rRNA gene Illumnia Sequencing (V3-V4 region) 341F (forward primer): 5′-CCTACGGGNGGCWGCAG-3′ 805R (reverse primer): 5′-GACTACHVGGGTATCTAATCC-3′ 806R (reverse primer): 5′-GGACTACHVGGGTWTCTAAT-3′

Table 6. Bacterial taxa identified across all studies

Genus	Species	NPC	Disease-associated*
Gram positive			
Lactobacillus			Dental caries
Streptococcus		Yes	Dental caries
Rothia	*mucilaginosa*	Yes	
Actinomyces		Yes	Dental caries
Oribacterium			
Gram negative			
Neisseria	*flavescens/subflava*	Yes	
Veillonella		Yes	Dental caries
Prevotella		Yes	Periodontitis
Fusobacterium			Periodontitis
Haemophilus	*parainfluenzae*	Yes	
Leptotrichia			Periodontitis
Alloprevotella			Periodontitis
Porphyromonas			Periodontitis
Kingella		Yes	
Lautropia			

A search was performed on Bacterial Feature Finder (http://csbg.cnb.csic.es/BaFF) to determine microbiological characteristics [39]. NPC, and disease status were determined based on recent findings in the literature [4,6–8,17,38]. NPC = nitrite-production capacity, meaning bacteria have been found to produce nitrite in the presence of nitrate [6]. *Disease-associated on a genus level. Note that some genera are clearly disease-associated (i.e. they generally increase in caries or periodontitis) but can contain health-associated species and strains, thus, future studies should test oral microbiome changes to at least species-level.

Table 7. Bacterial population increase versus decrease post NO_3^- supplementation.

Bacterial genera that increase	Percent of studies (n = 12)
Neisseria (incl. *flavescens* and *subflava* spp.)	67%
Rothia (incl. *mucilaginosa* sp.)	58%
Haemophilus	17%
Alloprevotella/Prevotella	8%
Veillonella	8%
Kingella	8%
Lautropia	8%
Streptococcus	8%
Bacterial genera that decrease	Percent of studies (n = 12)
Alloprevotella/Prevotella	33%
Veillonella	25%
Streptococcus	25%
Fusobacterium	8%
Oribacterium	8%
Porphyromonas	8%
Leptotrichia	8%
Actinomyces	8%
Haemophilus parainfluenza	8%
Neisseria subflava	8%

pH levels and/or enhancing lactic acid/lactate consumption [7,15,31,35,38]. These findings coincide with other studies which have shown acute increases in oral pH post NO_3^- consumption [18,47]. An increase in pH combined with a decrease in *Veillonella* and *Streptococcus* is a positive change from a caries perspective; *Streptococcus* is a carbohydrate fermenting genus whilst *Veillonella* consumes the lactic acid produced by *Streptococci*, thus causing a consistent increase in both genera in the supragingival plaque of individuals with caries [48]. Nevertheless, these genera also contain health-associated species (e.g. *S. dentisani)* that can decrease during caries development [49]. Future studies should explore the effects of NO_3^- on the oral microbiota at species level. Overall, oral bacteria consume protons and lactic acid during denitrification and nitrite reduction to ammonium which increases pH levels, limits oral acidification and inhibits the growth of cariogenic representatives [7,35,38].

Importantly, one study in this review emphasised that NO_3^- could be used as a therapeutic intervention for plaque control or periodontal treatment [17]. Jockel-Schneider *et al.*, [16,17] found lettuce juice consumption, coupled with gingival debridement, in PD patients reduced gingival inflammation and significantly altered the subgingival microbiome to benefit oral health; NR *Rothia* and *Neisseria* genera significantly increased. Changes were associated with a significant increase in mean salivary NO_3^- levels. Thus, NO_3^- supplementation should be explored as an adjunct therapy to treat periodontitis [17]. This idea is supported by a recent study in which subgingival plaque of PD patients was grown *in vitro* and NO_3^- decreased biofilm formation, the levels of periodontitis-associated species and the dysbiosis index [50]. Overall, NO_3^- alters the oral environment to benefit the host, which makes it a strong candidate as a prebiotic for oral health (|Figure 3, left side) [7,8,16,17].

Table 8. NO_3^- supplementation and oral community composition/activity.

Impact of NO_3^- supplementation on oral health	Studies (n = 12)	Ref.
Studies reporting an increase in NRB associated with eubiosis	11	[7,15,17,30–32,34–38]
Studies reporting a decrease in bacterial species associated with dysbiosis	5	[7,15,32,36,37]
Studies reporting an increase in NRB associated with eubiosis and decrease in bacterial species associated with dysbiosis	5	[7,15,32,36,37]
Studies reporting an increase in salivary pH levels and/or lactate consumption	5	[7,15,31,35,38]

Table 9. NO_3^- supplementation and other health outcomes.

NO_3^- and *in vivo* health effects	Studies (n = 12)	Ref.
NO_3^- and systemic health benefits (CV and cognitive)	5	[15,30,32,34,36]
NO_3^- as a treatment for oral disease	1	[17]
Antiseptic mouthwash exacerbates dysbiosis	1	[33]

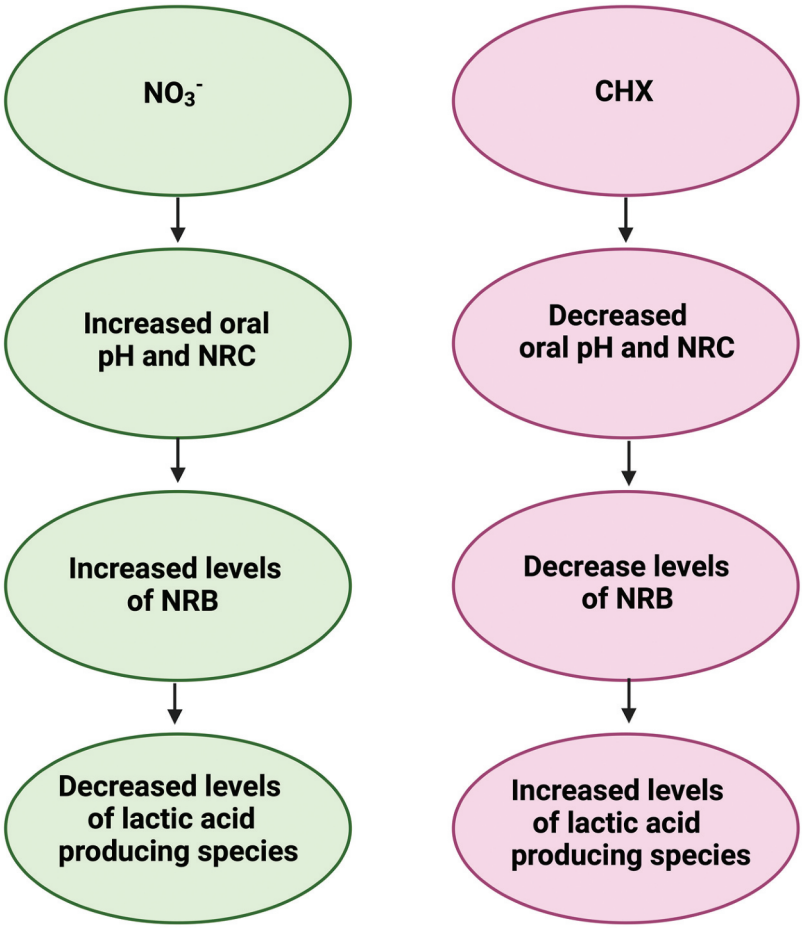

Figure 3. The effects of NO_3^- vs CHX on the oral environment: (left) summary based on findings in this review (right) summary based on (limited) current literature examining the effects of CHX on the oral environment (created with BioRender.com).

Advantages of NO_3^- supplementation over the use of chlorhexidine mouthwash

CHX inhibits plaque formation and exerts antibacterial action against various gram-positive and gram-negative bacteria [45,51,52]. In this review, Ashworth et al., [33] showed CHX mouthwash decreased NRC and oral pH, whilst significantly increasing salivary lactate and glucose levels. Other studies have reported similar findings (Figure 3, right side). Chatzigiannidou et al., [53] found that prolonged use of CHX-containing mouthwash altered salivary pH and increased lactic-acid producing species. Moreover, Bescos et al., [45] showed 7-day use of a CHX mouth rinse increased *Streptococcus* spp. abundance and reduced levels of *Actinomyces* spp., whilst also decreasing salivary pH and increasing lactate/glucose levels. Additionally, studies have shown CHX-containing mouth rinses are inducing resistance. Kulik *et al.*, [54] detected two- to fourfold increases in minimum inhibitory concentrations (MIC's) of *P. gingivalis* against sub-inhibitory concentrations of CHX after ≈ 30 passages. Wang *et al.*, [55] found CHX induced one to two-fold MIC increases in *F. nucleatum* and *P. gingivalis*. Thus, CHX-containing mouthwash can induce adaptation in oral bacteria and can also facilitate the development of cross-resistance to other antiseptics, including antibiotics, thus exacerbating AMR occurrence [10,12,52]. In contrast, NO_3^- exerts antibacterial action through NO_2^- and NO production, and resistance has not yet been observed [56]. Moreover, NO is significantly less toxic to human gingival fibroblasts than clinical concentrations of CHX [56,57]. Figure 3 compares and contrasts the effects of NO_3^- and CHX on the oral environment.

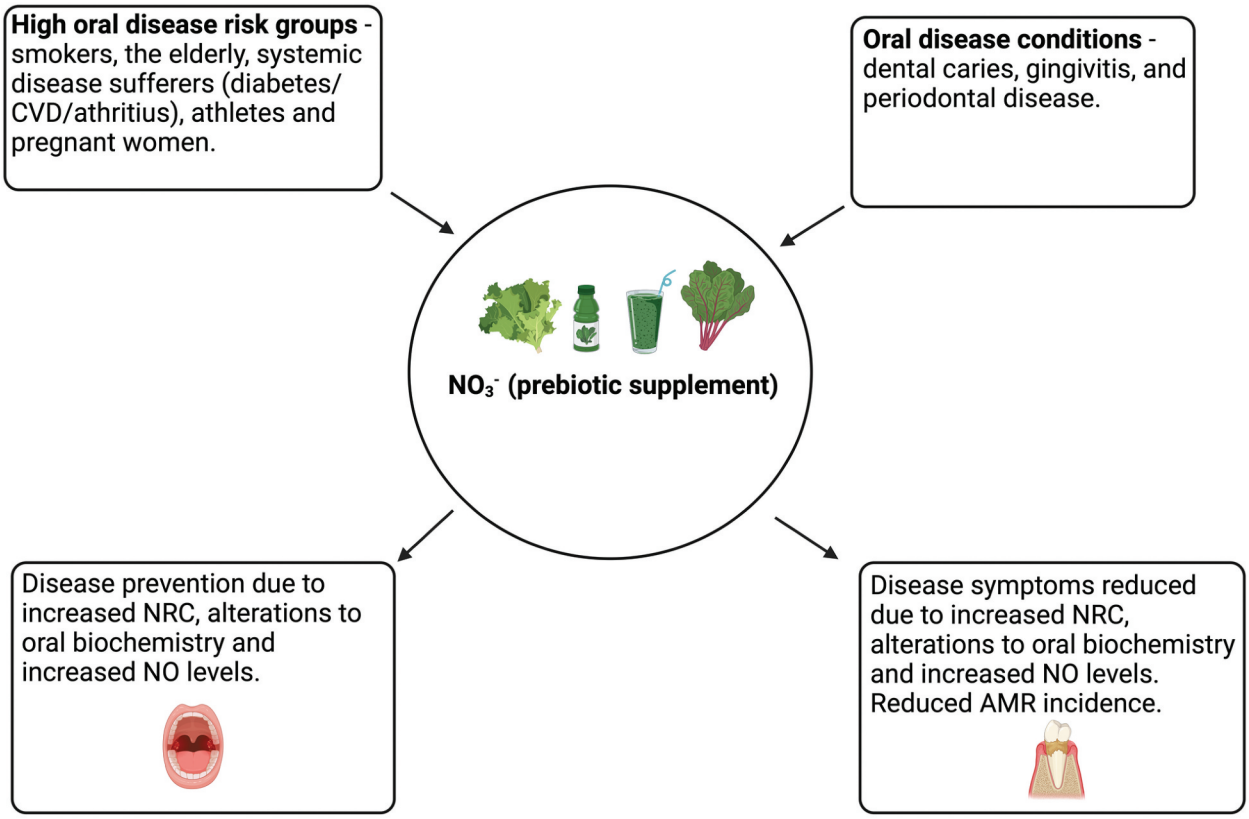

Figure 4. NO_3^- as a prebiotic for oral health. NO_3^- supplementation could be used in high risk groups to prevent oral disease (left side) or be used as a treatment method for individuals with oral disease(s) (right side) (created with BioRender.com).

NO_3^- and secondary findings

As discussed, NO_3^- reduction improves both oral and systemic health [1,4,6]. NO is a well-known vasodilator that decreases arterial stiffness and blood pressure [5,58–61]. The positive effects of NO on blood pressure have been well documented [1,3,4,40,62–64]. Webb et al., [62] showed a dose of beetroot juice (500ml) could significantly reduce systolic blood pressure (SBP) and diastolic blood pressure (DBP) by ≈ 10 mm Hg and ≈ 8 mm Hg, respectively. Moreover, Kenjale *et al.*, [63] showed beetroot juice could significantly reduce DBP in subjects with peripheral arterial disease vs placebo. Similarly, studies have shown NO has positive effects on vascular function. Endothelial dysfunction is associated with arterial stiffness and impaired blood flow [61,64]. Acute (2–6 hours; 68–583 mg) and chronic (7–42 days; 300–650 mg/d) NO_3^- consumption can significantly reduce arterial stiffness [59]. Additionally, another study showed consuming spinach soup for 7 days significantly reduced arterial stiffness and BP [65]. In contrast, CHX mouthwash has been shown to negate the BP lowering effects of NO_3^- [4].

In this review, five studies emphasised that oral microbiome changes were associated with cardiovascular health improvements, namely improvements to BP, mean arterial pressure (MAP), flow-mediated dilation (FMD), pulse wave velocity (PWV) and platelet aggregation [15,30,32,34,36]. Vanhatalo *et al.*, [32] showed that dietary NO_3^- supplementation reduced BP and improved MAP in older participants, whilst another clinical study suggested that NO_3^- sensitive microbial modules could serve as pre and probiotic targets to alter age-associated cardiovascular impairments [32,36]. A lowering of 5 mmHg in SBP can reduce the risk of CVD death caused by stroke and heart disease by 14% and 9%, respectively [5]. Furthermore, two studies reported improvements to FMD and PWV following NO_3^- consumption, whilst another trial recorded BP lowering effects [15,30,34]. Additionally, one study reported NO_3^- supplementation significantly lowered platelet aggregation [30]. Thus, evidence suggests dietary NO_3^- has cardioprotective benefits which are associated with oral microbiome-related changes post NO_3^- ingestion.

As previously highlighted, non-plant-based sources of NO_3^- and NO_2^- (processed meats and drinking water) can induce carcinogenesis and, in infants, increase the risk of methemoglobinemia [2,66]. A study in this review emphasised that organic sources of NO_3^- and NO_2^- can cause the formation of carcinogenic *N*-nitroso compounds (NOCs) linked to colorectal cancer [37]. However, high levels of antioxidants in vegetables counteract nitrosation [4]. Furthermore, an individual's daily NO_3^- intake is mostly derived from fruits and vegetables and generally exceeds acceptable daily intake levels (ADI's) (3.7mg NO_3^-/kg/day),

as established by The World Health Organisation [2,66]. Given the positive systemic and oral health benefits of inorganic NO_3^-, perhaps ADI's should be revaluated to reflect NO_3^-/NO_2^- source.

NO_3^-as a prebiotic for oral health

Throughout, connections between dietary NO_3^- consumption and positive oral health have been highlighted. In total, *n*=11 studies showed NO_3^- supplementation increased NR health-associated bacteria and/or reduced levels of common oral pathogens, thus establishing eubiosis and reducing oral disease risk [7,15,17,30–32,34–38]. Additionally, *n*=5 studies showed NO_3^- consumption modified oral biochemistry by increasing pH levels and reducing lactate production, thus also minimising dysbiosis [7,15,31,35,38]. Lastly, one study showed NO_3^- decreased gingival inflammation in patients with chronic gingivitis [16,17].

In contrast, evidence suggests CHX-containing mouthwashes used for periodontal treatment could stimulate dysbiosis and caries development, and could be exacerbating AMR [10,12,51,54,55]. Given the positive effects of vegetable-based sources of NO_3^- and the low risk of emerging NO resistance in oral pathogens [56], evidence in this review indicates NO_3^- could be used as an effective prebiotic to promote oral health. Overall, NO_3^- could serve as a preventative strategy against PD in high-risk groups or as a therapeutic intervention in individuals with PD (Figure 4). Although more studies are required to confirm the effects of NO_3^- and secondary metabolites on oral commensals, evidence provided in this review strongly indicates that NO_3^- could be used to simulate eubiosis and oral health.

Conclusion

This review has evaluated the impact of NO_3^- on the oral microbiome. The findings indicate that dietary NO_3^- increases oral health-associated bacterial genera, including *Rothia* and *Neisseria*, whilst reducing bacteria implicated in oral disease, including *Prevotella* and *Veillonella* spp. NO_3^- also beneficially alters the biochemical environment, by increasing oral pH and reducing dental caries risk. Additionally, the link between oral and systemic benefits was highlighted, particularly in relation to NO_3^- associated cardioprotective benefits. Further *in vivo* studies are required to uncover the mechanisms underlying the beneficial effects of NO_3^- on the oral microbiome; however, the evidence presented in this review indicates dietary NO_3^- could be used as a prebiotic for oral health.

Highlights

- This systematic review evaluated the effects of nitrate on the oral microbiome.
- Dietary nitrate and nitrate supplements increased oral health-associated genera, particularly *Rothia* and *Neisseria*.
- The genus *Prevotella*, which is normally associated with periodontal disease and halitosis, decreased post-nitrate consumption.
- Nitrate also increased oral pH, while decreasing the levels of *Streptococcus* and *Veillonella*, which is a positive change from a caries perspective.
- Oral health benefits were linked to systemic benefits, particularly improvements to markers of cardiovascular disease risk.
- Overall, nitrate could be considered as a prebiotic for oral health.

Disclosure statement

B.T. Rosier is a coinventor in a pending patent application owned by the FISABIO Institute, which protects the use of nitrate as a prebiotic and certain nitrate-reducing bacteria as probiotics. The remaining authors declare no competing interests.

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References

[1] Shannon OM, Easton C, Shepherd AI, et al. Dietary nitrate and population health: a narrative review of the translational potential of existing laboratory studies. BMC Sports Sci Med Rehabil. 2021;13(1):1–17. doi: 10.1186/s13102-021-00292-2

[2] Martin K. Dietary nitrates, nitrites, and food safety: Risks versus benefits. Act Scie Nutr. 2021;5(6):65–76. doi: 10.31080/ASNH.2020.05.0884

[3] Stanaway L, Rutherfurd-Markwick K, Page R, et al. Performance and health benefits of dietary nitrate supplementation in older adults: a systematic review. Nutrients. 2017;9(11):1171. doi: 10.3390/nu9111171

[4] Bryan NS, Burleigh MC, Easton C. The oral microbiome, nitric oxide and exercise performance. Nitric Oxide. 2022;125-126:23–30. doi: 10.1016/j.niox.2022.05.004

[5] McDonagh STJ, Wylie LJ, Thompson C, et al. Potential benefits of dietary nitrate ingestion in healthy and clinical populations: a brief review. Eur J Sport Sci. 2019;19(1):15–29. doi: 10.1080/17461391.2018.1445298

[6] Rosier BT, Takahashi N, Zaura E, et al. The importance of nitrate reduction for oral health. J Dent Res. 2022;101(8):887–897. doi: 10.1177/00220345221080982

[7] Rosier BT, Buetas E, Moya-Gonzalvez EM, et al. Nitrate as a potential prebiotic for the oral microbiome. Sci Rep. 2020;10(1). doi: 10.1038/s41598-020-69931-x

[8] Morou-Bermúdez E, Torres-Colón JE, Bermúdez NS, et al. Pathways linking oral bacteria, nitric oxide

metabolism, and health. J Dent Res. 2022;101 (6):623–631. doi: 10.1177/00220345211064571
[9] Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim). 2017;11(2):72–80.
[10] Saleem HG, Seers CA, Sabri AN, et al. Dental plaque bacteria with reduced susceptibility to chlorhexidine are multidrug resistant. BMC Microbiol. 2016;16 (1):1–9. doi: 10.1186/s12866-016-0833-1
[11] Paul O, Arora P, Mayer M, et al. Inflammation in periodontal disease: possible link to vascular disease. Front Physiol. 2021;11. doi: 10.3389/fphys.2020.609614
[12] Verspecht T, Rodriguez Herrero E, Khodaparast L, et al. Development of antiseptic adaptation and cross-adaptation in selected oral pathogens in vitro. Sci Rep. 2019;9(1). doi: 10.1038/s41598-019-44822-y
[13] Murray CJL, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet (London, England). 2022;399(10325):629–655.
[14] Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309–318. doi: 10.1179/2047773215Y.0000000030
[15] Burleigh M, Liddle L, Muggeridge DJ, et al. Dietary nitrate supplementation alters the oral microbiome but does not improve the vascular responses to an acute nitrate dose. Nitric Oxide. 2019;89:54–63. doi: 10.1016/j.niox.2019.04.010
[16] Jockel-Schneider Y, Goßner SK, Petersen N, et al. Stimulation of the nitrate-nitrite-NO-metabolism by repeated lettuce juice consumption decreases gingival inflammation in periodontal recall patients: a randomized, double-blinded, placebo-controlled clinical trial. J Clinic Periodontol. 2016;43 (7):603–608. doi: 10.1111/jcpe.12542
[17] Jockel-Schneider Y, Schlagenhauf U, Stölzel P, et al. Nitrate-rich diet alters the composition of the oral microbiota in periodontal recall patients. J Periodontol. 2021;92(11):1536–1545. doi: 10.1002/JPER.20-0778
[18] Hohensinn B, Haselgrübler R, Müller U, et al. Sustaining elevated levels of nitrite in the oral cavity through consumption of nitrate-rich beetroot juice in young healthy adults reduces salivary pH. Nitric Oxide. 2016;60:10–15. doi: 10.1016/j.niox.2016.08.006
[19] PRISMA. PRISMA 2020 checklist; 2021 [cited 2022 Nov 1]. Available from: http://www.prisma-statement.org
[20] Scottish Intercollegiate Guidelines Network. Algorithm for classifying study design for questions of effectiveness; 2021 [cited 2022 Nov 2022]. Available from: https://www.sign.ac.uk/assets/study_design.pdf
[21] University of Strathclyde. *Systematic Review Steps*; 2022 [cited 2022 Nov 1]. Available from: https://guides.lib.strath.ac.uk/systematic/steps
[22] Centre for Evidence-Based Management. Critical Appraisal Of a Controlled Study; 2019 [cited 2022 Nov 2]. Available from: https://cebma.org/resources-and-tools/what-is-critical-appraisal/
[23] Critical Appraisal Skills Programme. CASP Randomised Controlled Trial Checklist; 2022 [cited 2022 Nov 2]. Available from: https://casp-uk.net/casp-tools-checklists/
[24] Greenhalgh T, Robert G, Bate P, et al. Diffusion of innovations in health service organisations a systematic literature review: appendix 2, critical appraisal checklists. London: BMJ. 2005.
[25] The Joanna Briggs Institute. *Checklist For Quasi-Experimental Studies*; 2022 [cited 2022 Nov 2]. Available from: https://jbi.global/critical-appraisal-tools
[26] Centre for Evidence-Based Management. *Critical Appraisal Checklist For Cross-Sectional Study*; 2014 [cited 2022 Nov 2]. Available from: https://cebma.org/wp-content/uploads/Critical-Appraisal-Questions-for-a-Cross-Sectional-Study-July-2014-1.pdf
[27] Strengthening the reporting of observational studies in epidemiology. STROBE Checklist For Cross-Sectional Studies; 2022 [cited 2022 Nov 2]. Available from: https://www.strobe-statement.org/checklists/
[28] University of Exeter Library. Checklist For In Vitro Studies; 2022 [cited 2022 Nov 2]. Available from: https://libguides.exeter.ac.uk/c.php?g=660317&p=4708597
[29] Sheth VH, Shah NP, Jain R, et al. Development and validation of a risk-of-bias tool for assessing in vitro studies conducted in dentistry: the QUIN. J Prosthet Dent. 2022. doi: 10.1016/j.prosdent.2022.05.019
[30] Velmurugan S, Gan JM, Rathod KS, et al. Dietary nitrate improves vascular function in patients with hypercholesterolemia: a randomized, double-blind, placebo-controlled study. Am J Clin Nutr. 2016;103 (1):25–38. doi: 10.3945/ajcn.115.116244
[31] Koopman JE, Buijs MJ, Brandt BW, et al. Nitrate and the origin of saliva influence composition and short chain fatty acid production of oral microcosms. Microb Ecol. 2016;72(2):479–492. doi: 10.1007/s00248-016-0775-z
[32] Vanhatalo A, Blackwell JR, L'Heureux JE, et al. Nitrate-responsive oral microbiome modulates nitric oxide homeostasis and blood pressure in humans. Free Radic Biol Med. 2018;124:21–30. doi: 10.1016/j.freeradbiomed.2018.05.078
[33] Ashworth A, Cutler C, Farnham G, et al. Dietary intake of inorganic nitrate in vegetarians and omnivores and its impact on blood pressure, resting metabolic rate and the oral microbiome. Free Radic Biol Med. 2019;138:63–72. doi: 10.1016/j.freeradbiomed.2019.05.010
[34] Liddle L, Burleigh MC, Monaghan C, et al. Variability in nitrate-reducing oral bacteria and nitric oxide metabolites in biological fluids following dietary nitrate administration: An assessment of the critical difference. Nitric Oxide. 2019;83:1–10. doi: 10.1016/j.niox.2018.12.003
[35] Rosier BT, Moya-Gonzalvez EM, Corell-Escuin P, et al. Isolation and Characterization of Nitrate-Reducing Bacteria as Potential Probiotics for Oral and Systemic Health. Front Microbiol. 11. doi: 10.3389/fmicb.2020.555465
[36] Vanhatalo A, L'Heureux JE, Kelly J, et al. Network analysis of nitrate-sensitive oral microbiome reveals interactions with cognitive function and cardiovascular health across dietary interventions. Redox Biol. 2021;41:101933. doi: 10.1016/j.redox.2021.101933
[37] Sinha R, Zhao N, Goedert JJ, et al. Effects of processed meat and drinking water nitrate on oral and fecal microbial populations in a controlled feeding study. Environ Res. 2021;197:111084. doi:10.1016/j.envres.2021.111084
[38] Rosier BT, Palazón C, García-Esteban S, et al. A single dose of nitrate increases resilience against acidification derived from sugar fermentation by the oral

microbiome. Front Cell Infect Microbiol. 2021;11:692883. doi: 10.3389/fcimb.2021.692883
[39] López-Ibáñez J, Martín LT, Chagoyen M, et al. Bacterial feature finder (BaFF)-a system for extracting features overrepresented in sets of prokaryotic organisms. Bioinformatics (Oxford Academia). 2019;35 (18):3482–3483. doi: 10.1093/bioinformatics/btz099
[40] Pignatelli P, Fabietti G, Ricci A, et al. How periodontal disease and presence of nitric oxide reducing oral bacteria can affect blood pressure. Int J Mol Sci. 2020;21(20):7538. doi: 10.3390/ijms21207538
[41] Radaic A, Kapila YL. The oralome and its dysbiosis: new insights into oral microbiome-host interactions. Computat Struct Biotechnol J. 2021;19:1335–1360. doi: 10.1016/j.csbj.2021.02.010
[42] Miyoshi T, Oge S, Nakata S, et al. *Gemella haemolysans* inhibits the growth of the periodontal pathogen porphyromonas gingivalis. Sci Rep. 2021;11(1). doi: 10.1038/s41598-021-91267-3
[43] Zhou P, Manoil D, Belibasakis GN, et al. Veillonellae: beyond bridging species in oral biofilm ecology. Front Oral Health. 2021;2. doi: 10.3389/froh.2021.774115
[44] Acar B, Çağlayan F, İnkaya AÇ, et al. *Actinomyces*-associated lesions located in the gingiva: case report of rare gingival lesions. Contemp Clin Dent. 2017;8 (1):182–184. doi: 10.4103/0976-237X.205067
[45] Bescos R, Ashworth A, Cutler C, et al. Effects of chlorhexidine mouthwash on the oral microbiome. Sci Rep. 2020;10(1). doi: 10.1038/s41598-020-61912-4
[46] Liu T, Chen Y-C, Jeng S-L, et al. Short-term effects of chlorhexidine mouthwash and Listerine on oral microbiome in hospitalized patients. Front Cell Infect Microbiol. 2023;13:13. doi: 10.3389/fcimb.2023.1056534
[47] Burleigh MC, Sculthorpe N, Henriquez FL, et al. Nitrate-rich beetroot juice offsets salivary acidity following carbohydrate ingestion before and after endurance exercise in healthy male runners. PLoS One. 2020;15(12): e0243755. doi: 10.1371/journal.pone.0243755
[48] Mashima I, Nakazawa F, O'Toole GA. The interaction between streptococcus spp. And veillonella tobetsuensis in the early stages of oral biofilm formation. J Bacteriol. 2015;197(13):2104–2111. doi: 10.1128/JB.02512-14
[49] López-Santacruz HD, López-López A, Revilla-Guarinos A, et al. Streptococcus dentisani is a common inhabitant of the oral microbiota worldwide and is found at higher levels in caries-free individuals. Int Microbiol. 2021;24(4):619–629. doi: 10.1007/s10123-021-00222-9
[50] Mazurel D, Carda-Diéguez M, Langenburg T, et al. Nitrate and a nitrate-reducing Rothia aeria strain as potential prebiotic or synbiotic treatments for periodontitis. NPJ Biofilms Microbiomes. 2023;9 (1):40. doi: 10.1038/s41522-023-00406-3
[51] Pałka Ł, Nowakowska-Toporowska A, Dalewski B. Is chlorhexidine in Dentistry an Ally or a foe? A narrative review. Healthcare. 2022;10(5):764. doi: 10.3390/healthcare10050764
[52] Brookes ZLS, Belfield LA, Ashworth A, et al. Effects of chlorhexidine mouthwash on the oral microbiome. J Dent. 2021;113:103768. doi: 10.1016/j.jdent.2021.103768
[53] Chatzigiannidou I, Teughels W, Van de Wiele T, et al. Oral biofilms exposure to chlorhexidine results in altered microbial composition and metabolic profile. NPJ Biofilms Microbiomes. 2020;6(1):13. doi: 10.1038/s41522-020-0124-3
[54] Kulik EM, Waltimo T, Weiger R, et al. Development of resistance of mutans streptococci and porphyromonas gingivalis to chlorhexidine digluconate and amine fluoride/stannous fluoride-containing mouthrinses, in vitro. Clin Oral Invest. 2015;19(6):1547–1553. doi: 10.1007/s00784-014-1379-y
[55] Wang S, Wang H, Ren B, et al. Do quaternary ammonium monomers induce drug resistance in cariogenic, endodontic and periodontal bacterial species? Dental Mater. 2017;33(10):1127–1138. doi: 10.1016/j.dental.2017.07.001
[56] Nambu T, Wang D, Mashimo C, et al. Nitric Oxide Donor Modulates a Multispecies Oral Bacterial Community—An In Vitro Study. Microorganisms. 2019;7(9):353. doi: 10.3390/microorganisms7090353
[57] Backlund CJ, Sergesketter AR, Offenbacher S, et al. Antibacterial efficacy of exogenous nitric oxide on periodontal pathogens. J Dent Res. 2014;93 (11):1089–1094. doi: 10.1177/0022034514529974
[58] Naseem KM. The role of nitric oxide in cardiovascular diseases. Mol Aspect Med. 2005;26(1–2):33–65. doi: 10.1016/j.mam.2004.09.003
[59] Bondonno CP, Blekkenhorst LC, Liu AH, et al. Vegetable-derived bioactive nitrate and cardiovascular health. Mol Aspect Med. 2018;61:83–91. doi: 10.1016/j.mam.2017.08.001
[60] Fadel PJ. Nitric oxide and cardiovascular regulation. Hypertension. 2017;69(5):778–779. doi: 10.1161/HYPERTENSIONAHA.117.08999
[61] Infante T, Costa D, Napoli C. Novel insights regarding nitric oxide and cardiovascular diseases. Angiology. 2021;72(5):411–425. doi: 10.1177/0003319720979243
[62] Webb AJ, Patel N, Loukogeorgakis S, et al. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension. 2008;51(3):784–790. doi: 10.1161/HYPERTENSIONAHA.107.103523
[63] Kenjale AA, Ham KL, Stabler T, et al. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. J Appl Physiol. 2011;110 (6):1582–1591. doi: 10.1152/japplphysiol.00071.2011
[64] Ivy JL. Inorganic Nitrate Supplementation for Cardiovascular Health. Methodist DeBakey Cardiovasc J. 2019;15(3):200–206. doi: 10.14797/mdcj-15-3-200
[65] Jovanovski E, Bosco L, Khan K, et al. Effect of spinach, a high dietary nitrate source, on arterial stiffness and related hemodynamic measures: a randomized, controlled trial in healthy adults. Clin Nutr Res. 2015;4 (3):160–167. doi: 10.7762/cnr.2015.4.3.160
[66] Bedale W, Sindelar JJ, Milkowski AL. Dietary nitrate and nitrite: Benefits, risks, and evolving perceptions. Meat Sci. 2016;120:85–92. doi: 10.1016/j.meatsci.2016.03.009