# Human gut microbiome and food additives: a critical review of the evidence

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## 1. Introduction

The human gastrointestinal tract is colonized by a complex consortium of several trillion microbial cells (microbiota), which interact closely with the host along the 32 $m^2$ area [1] of the luminal layer. The gut microbiota play an important role in supporting human health, including maintenance of immune homeostasis, nutritional and metabolic balance, and preventing pathogen colonization in the gut [2–4]. Disruption of the gut microbiota, on the other hand, is implicated in a growing number of allergic, metabolic, and chronic inflammatory diseases, due to the consequences that microbial perturbations have on crucial host systems.

Recent evidence suggests that some food additives (including artificial sweeteners and some emulsifiers) may have a destabilizing effect on the gut microbiota and host-microbial interactions, with cascading effects on intestinal homeostasis and human metabolic health [5–9]. That said, the observed effects of food additives on the gut microbiota often lack consensus, with both beneficial and adverse outcomes reported in literature. As industrialization spreads globally, transforming food systems across the world, human diets include more processed foods and various food additives [10–12]. Accordingly, there is an emerging interest in understanding potential sources of variation and discrepancy among studies examining the impacts of food additives on the gut microbiota.

Part of the inconsistency in the literature is related to the large variety of different methods that are commonly used to study the composition of the microbiota and its functional activities. This includes cultivation-based approaches, methods for untargeted characterization of diversity and composition of microbial communities (most commonly with DNA sequencing technologies), as well as methods used to evaluate functional behavior of microbiome. Moreover, microbiome studies can differ substantially in experimental design (e.g., sampling strategy, host species, host sex, sample type, etc.) and analytical procedures [13], which may further limit opportunities for a synthesis of how specific food additives impact the gut microbiota and host health. Finally,

differences in dose vary widely in the literature, leading to further inconsistencies among studies.

This report provides a critical review of the evidence to assess (1) the potential for different food additives to impact human health via changes in the gut microbiome, with a particular focus on emulsifiers and artificial sweeteners [14,15], (2) appropriate methodologies for microbiota analysis and their informative value for the risk assessment of food additives, and (3) impacts of food additives on the gut microbiota and host health based on study outcomes via a qualitative meta-analysis of relevant articles.

# 2. Methods

## 2.1. Literature search

A list of keywords was constructed for searching relevant research articles on the impacts of food additives on the gut microbiota in PubMed database (using PubMed advanced search builder, https://pubmed.ncbi.nlm.nih.gov/advanced/). We focused on ingredients within two functional classes of food additives, *sweeteners* and *emulsifiers* (including emulsifying salts), as defined by the international food standards maintained by the World Health Organization and Food and Agriculture Organization of the United Nations (i.e., Codex Alimentarius, document CXG 36-1989 [16]). Every food additive belonging to one of these classes was included in the search terms, resulting in 126 emulsifiers and 25 sweeteners. The complete list of food additives and their respective INS E numbers (i.e., International Numbering System proposed by Codex Alimentarius) used to search published records in PubMed is provided in Table S1 (see *Appendix 1*).

We used a two-step approach to retrieve all relevant research articles published between the years 2002 and 2022 (i.e., last 20 years; see Figure 1). First, every ingredient was queried individually with the addition of the following gut microbiome-specific search terms: ‘AND (intestinal-microbi* OR gut-microbi* OR gastrointestinal-microbi* OR intestinal-microflora OR gastrointestinal-microflora OR gut-microflora) AND (2002:2022[pdat]) AND journal article NOT (review OR systematic review)’. This search identified food additives without any published evidence (i.e., without records on PubMed), and potentially represent knowledge gaps. After this step, all ingredients with at least one published article were queried in combination with their respective INS E number and gut microbiome-specific search terms (as above) for performing a critical literature search. This search was conducted separately for sweeteners and emulsifiers. The exact search terms used to search published records in PubMed are provided in the *Appendix 1*.

In a second step, we performed a complementary search using more generic terms describing sweeteners and emulsifiers to account for any potential variation in definitions among studies (Figure 1, *Appendix 1*). For example, in addition to the gut microbiome-specific keywords, search terms included ‘(artificial-sweetener OR artificial-sweeteners OR non-caloric-sweetener OR non-nutritive-sweetener)’ and ‘(emulsifier OR surfactant)’, for sweeteners and emulsifiers

respectively. Finally, the resulting tables from these two steps were merged and duplicates were removed prior to any downstream analysis. This literature search was last updated in November 2022 and yielded a total of 1088 and 2557 articles for sweeteners and emulsifiers, respectively (Figure 1). For an overview of the references, see supplementary information tables in the Supplementary Data package.

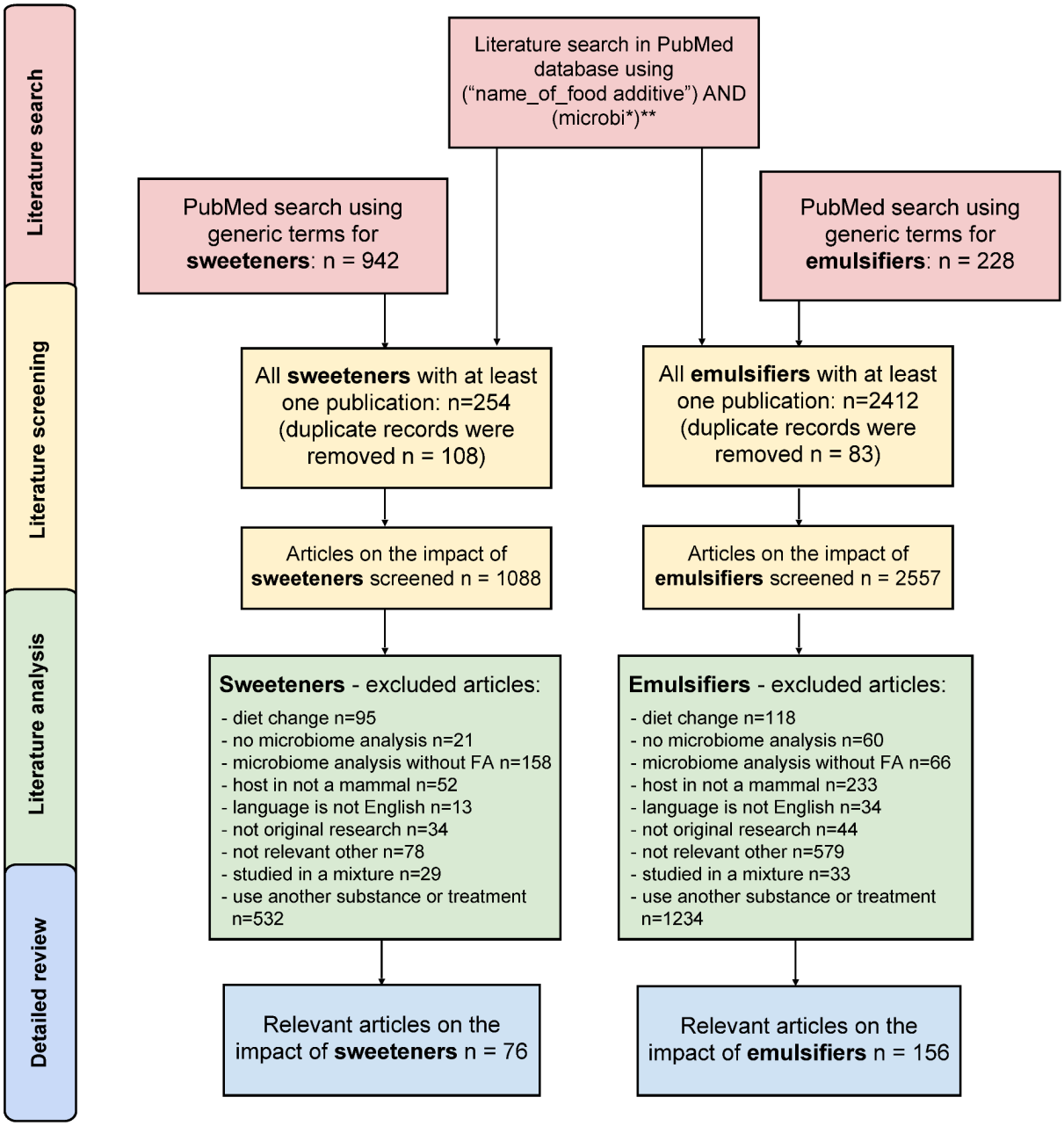


**Figure 1**. Overview of a critical literature review (**for a detailed list of gut microbiome-specific search terms see *Section 2.1*; for an overview of the references, see supplementary information tables in the Supplementary Data package; FA refers to a food additive).

## 2.2. Literature analysis

All *titles* and *abstracts* of the resulting articles were reviewed manually to remove articles that are out of scope and/or those that are not original research. The minimum inclusion criteria were as follows: (i) the article is written is English, (ii) original research (e.g., not review or perspective articles), (iii) ingredient is not administered in a complex mixture (except for specific products with a defined formula, such as Splenda), (iv) host species is a mammal. This initial quality-control screening substantially reduced the number of relevant articles, with 76 articles for sweeteners and 156 articles for emulsifiers available for further analysis (Figure 1). For an overview of the references, see supplementary information tables in the Supplementary Data package. After this step, all *methods* and *results* sections in the remaining articles were further inspected to document study details, such as (i) ingredient evaluated, (ii) study design (e.g., cross-sectional, longitudinal), (iii) host species, (iv) host tissue/sample type (e.g., ileum, cecum, stool, etc.), (v) host sex, and (vi) microbiota methodology (see *Section 2.3*, and Supplementary Data package).

## 2.3. Classification of microbiota methodology

All relevant articles were inspected to characterize methodological approaches used to study the impacts of food additives on the gut microbiota and its functional activities (e.g., cultivation-based approaches, quantitative PCR, marker-gene amplicon sequencing, shotgun metagenome sequencing, metabolomics, etc.). Methods were classified regarding their detection limits and informative value for the risk assessment of food additives. Articles in which gut microbiota were examined with untargeted sequencing-based approaches (see Section 3.3) were further classified with respect to the technology used (e.g., marker-gene amplicon sequencing, shotgun metagenome sequencing, metatranscriptomics), including sequencing platform type and instrument model/data type. For these articles, we also assessed the volume of associated sequence data in public data repositories that could potentially be used for a meta-analysis of published data. We manually screened relevant articles and verified the availability/suitability of available (if any) published datasets for a quantitative meta-analysis.

## 2.4. Meta-analysis of published studies

We also conducted a qualitative meta-analysis of relevant articles, summarizing the impacts of food additives on the gut microbiota and host health based on study outcomes. Note that such qualitative meta-analysis was conducted only for articles in which gut microbiota were examined with untargeted sequencing-based approaches, and only for ingredients with at least three published articles.

We extracted information on the impacts of food additives on the gut microbiota, and classified changes (if any) in key microbiome metrics, such as (i) alpha diversity (i.e., the biodiversity within individual samples; see *Section 3.3* for more details) and (ii) beta diversity (i.e., dissimilarity in biodiversity between samples), using the following terms: *change*, *no change*, *inconclusive*, and *no details* (i.e., no information provided by the original authors). Similarly, (iii)

overall impacts on host health were classified as *negative*, *positive*, *no effect*, *inconclusive*, and *no details*. For an overview of the references, see supplementary information tables in the Supplementary Data package.

A quantitative meta-analysis of published study data had been planned. However, for the majority of studies raw datasets are not openly available (see *Section 3.3*, Supplementary Data package). Hence, we focus instead on qualitative comparisons of the literature.

# 3. Results and discussion

## 3.1. Trends revealed by a critical literature review

We identified 76 relevant research articles on the impacts of sweeteners on the gut microbiota in the PubMed database. For food emulsifiers, we identified 156 relevant articles (Figure 1). These articles were manually inspected to document study details, identify potential knowledge gaps, and to examine important research trends. From the 25 sweeteners included in the search terms (see *Section 2.1*), 18 sweeteners had at least one relevant publication, five sweeteners had no records on PubMed (Table 1A), and two more (i.e., alitame and thaumatin) were removed from further analysis due to the lack of publications matching the minimum inclusion criteria (see *Section 2.2*). From the 126 emulsifiers listed in the Codex Alimentarius, we analyzed 116 ingredients, as three emulsifiers were analyzed together with sweeteners due to their dual classification (i.e., maltitol, lactitol, xylitol) and seven others were grouped together as 'polysorbates' (i.e., emulsifiers: E430-E436). From these, only 32 emulsifiers had at least one publication record that met the minimum inclusion criteria, whereas more than one third of emulsifiers (n=46) had no records on PubMed (Table 1B). Notably, some of the sweeteners and emulsifiers that lack any published evidence on the impacts on the gut microbiota and its functional activities may also have the potential to disturb intestinal homeostasis, affecting the gut microbiota activities and host health. The implication is that these ingredients represent important knowledge gaps that should be addressed in future studies.

**Table 1**. Food additives and their respective INS E numbers (International Numbering System) without published records on PubMed.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **(A) Sweeteners** without published records on PubMed | | | | | | | |
| **960b** | Steviol glycosides from fermentation | **960d** | Glucosylated steviol glycosides | **964** | Polyglycitol syrup | | |
| **960c** | Enzymatically produced steviol glycosides | **962** | Aspartame-acesulfame salt | | | | |
| **(B) Emulsifiers** without published records on PubMed | | | | | | | |
| **326** | Potassium lactate | **444** | Sucrose acetate isobutyrate | **474** | Sucroglycerides | **902** | Candelilla wax |
| **335ii** | Sodium tartrates | **445** | Glycerol ester of rosin | **475** | Polyglycerol esters of fatty acids | **999ii** | Quillaia extract type 2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **336** | Potassium tartrates | **464** | Hydroxypropyl methyl cellulose | **477** | Propylene glycol esters of fatty acids | **1401** | Acid-treated starch |
| **341** | Calcium dihydrogen phosphate | **467** | Ethyl hydroxyethyl cellulose | **479** | Thermally oxidized soya bean oil interacted with mono- and diglycerides of fatty acids | **1403** | Bleached starch |
| **343i** | Magnesium dihydrogen phosphate | **471** | Mono- and di-glycerides of fatty acids | **484** | Stearyl citrate | **1420** | Starch acetate |
| **403** | Ammonium alginate | **471a** | Acetic and fatty acid esters of glycerol | **485** | Sodium stearoyl fumarate | **1422** | Acetylated distarch adipate |
| **407a** | Processed eucheuma seaweed (PES) | **472b** | Lactic and fatty acid esters of glycerol | **486** | Calcium stearoyl fumarate | **1440** | Hydroxypropyl starch |
| **419** | Gum ghatti | **472d** | Tartaric acid esters of mono- and diglycerides of fatty acids | **488** | Ethoxylated mono- and diglycerides | **1442** | Hydroxypropyl distarch phosphate |
| **423** | Octenyl succinic acid (OSA) modified gum arabic | **472e** | Diacetyltartaric and fatty acid esters of glycerol | **492** | Sorbitan tristearate | **1451** | Acetylated oxidized starch |
| **427** | Cassia gum | **472g** | Succinylated monoglycerides | **493** | Sorbitan monolaurate | **1518** | Triacetin |
| **441** | Superglycerinate hydrogenated rapeseed oil | **473** | Sucrose esters of fatty acids | **495** | Sorbitan monopalmitate | | |
| **442** | Ammonium salts of phosphatidic acid | **473a** | Sucrose oligoesters, type I and type II | **542** | Bone phosphate | | |

We found substantial variation in research efforts among 18 sweeteners and 32 emulsifiers with at least one relevant publication, such that the total numbers of records were not evenly distributed among ingredients studied (Figure 2). For example, sucralose (n=28), saccharine (n=19), steviol glycosides (n=17), aspartame (n=15), and acesulfame-K (n=11) were among the top five sweeteners based on the number of records retrieved (Figure 2A). A number of studies have also examined lactitol (n=10), xylitol (n=7), and glycyrrhizin (n=4), likely due to their putative beneficial health effects. Similarly, the volume of available literature on food emulsifiers differed substantially among ingredients, with the number of records decreasing rapidly to form a long tail of emulsifiers with only one or two published studies (Figure 2B). Based on the number of records in reviewed literature, the most popular emulsifiers were pectins (n=36), guar gum (n=26), starches (n=16), polysorbates (n=16), sodium alginate (n=11), cellulose (n=11), dextrins (n=10), carrageenan (n=9), sodium carboxymethyl cellulose (n=7), and tannic acid (n=7). In the *Appendix 1* we provide a visual summary of results of a qualitative meta-analysis for all relevant sweeteners and emulsifiers (see *Section 2.4* for methods), i.e., summarizing the impacts of food additives on the gut microbiota and host health based on study outcomes (Figures S1 and S2). In the detailed review and discussion below, we focus only on those sweeteners and emulsifiers with at least some reports of adverse health effects, or with contradictory results, and on those most commonly used in dietary products (*Section 3.2*). Note that in this following section (and throughout the report), the comparison of doses in animal studies to the Acceptable Daily Intake (ADI) are used only for illustrative purposes (rather than

in the context of reference values), as while the ADI is derived from animal studies it represents the ADI considered safe for humans and, thus should only be used to directly compare human exposure.

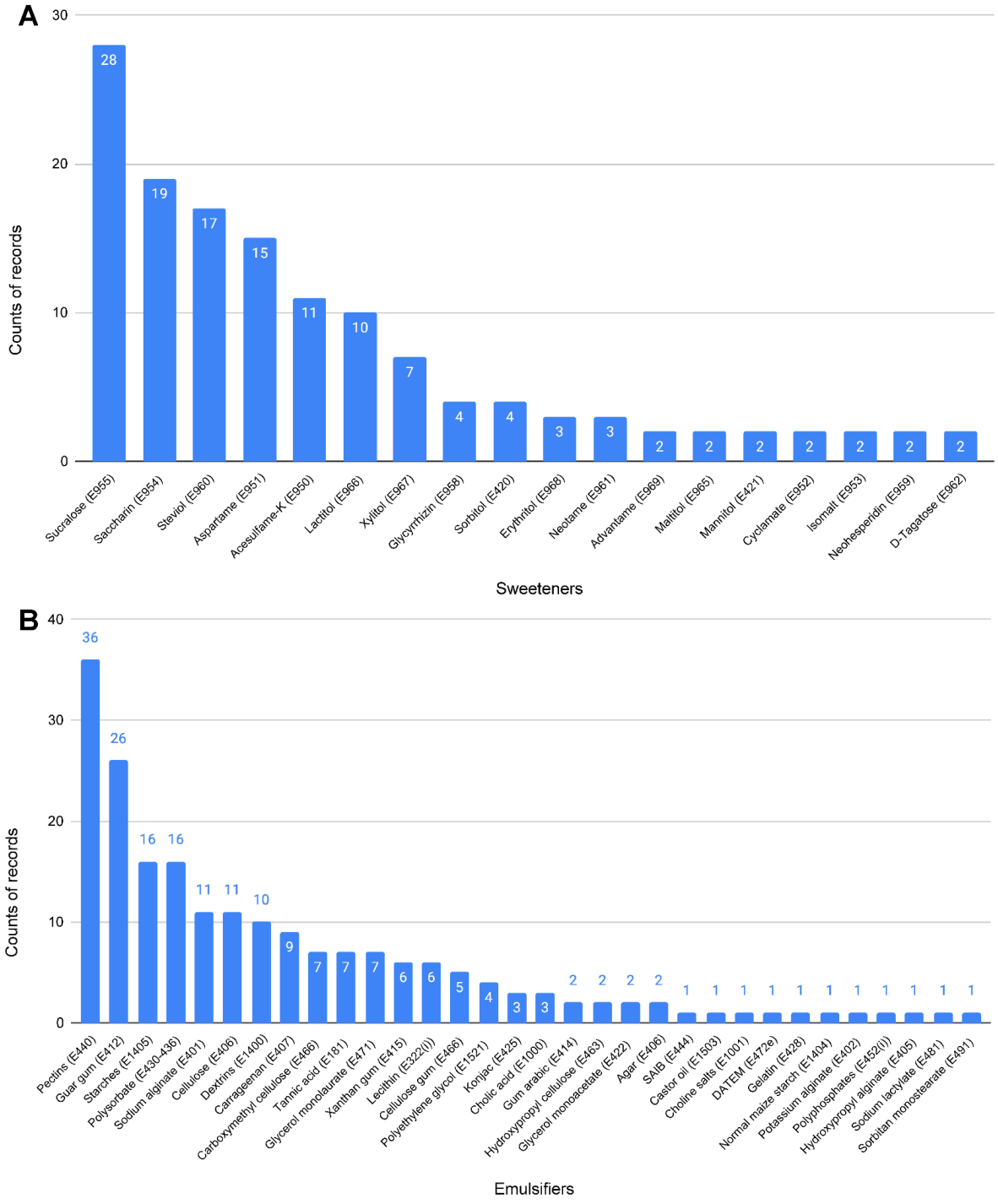


**Figure 2**. Counts of records examining the impact of food additives (A) sweeteners and (B) emulsifiers on the gut microbiota (based on the reviewed literature: n=76 articles for sweeteners, and n=156 for emulsifiers).

## 3.2. Classification of food additives and their hypothetical impacts on microbiome-host interactions

### *Sweeteners*

Sweeteners are any food additive that increases the perceived sweetness of a food, and can be further classified into nutritive and non-nutritive sweeteners (NNS). Both groups have sweeteners from synthetic and natural origin [15]. Nutritive sweeteners include sugars and sugar alcohols such as xylitol [17]. NNS are characterized by their negligible caloric value and high sweetening capacity. Some NNSs derive their low caloric value due to their very high sweetening value, and hence very low amounts are needed for sweetening foods, reducing the likelihood of harmful side-effects if their metabolites are readily absorbed and non-toxic. However, some other NNSs cannot be metabolized by humans, lending their low caloric value (and hence benefits, e.g., to those with diabetes mellitus or other metabolic disorders). These sweeteners then pass undigested into the intestine, where they can be metabolized by the gut microbiota, raising concerns of unintended side-effects. The primary focus in the literature is on NNSs with potential impact on gut microbiota metabolism, although the precise mechanisms by which this can further cascade to impact human health are yet to be described. Other relevant reviews have been published on gut-microbiota effects of these and other sweeteners [18,19].

**Cyclamate (E952)** was the first sweetener with demonstrated impacts on microbial metabolism in the gut. In humans and various species of lab animals, cyclamate is metabolized by intestinal bacteria to form cyclohexylamine, a toxic compound [20–22]. Subchronic exposure to cyclamate appears to induce metabolic adaptation independent of taxonomic shifts in an *in vitro* system, leading to increased metabolic conversion to cyclohexylamine [23]. However, the reported molar conversion rates are low (≤ 3%), making it unlikely for cyclamate consumption within the ADI to lead to accumulation of cyclohexylamine at toxic levels. Cyclamate remains banned for use in foods in the USA, yet it is a legal food additive in the EU and Switzerland. Cyclamate has not been studied using more recent methods for profiling microbiota and microbial metabolism (e.g., see *Section 3.3*), warranting re-examination.

**Acesulfame potassium (acesulfame K; E950)** was associated with changes to gut microbiota diversity, composition, and also adverse impacts on host health in nearly half (43%, total n=7 studies) of the records retrieved. These studies noted significant changes in the gut microbiota of mice fed acesulfame K (at 1, 2.5, and 10 times the ADI), with associated metabolic and/or immunological changes [24–26], but these effects were not replicated in rats fed ADIx2.5 [27] or in mice fed ADIx1 [28]. In one study, intestinal dysbiosis induced by acesulfame K (ADIx1) in pregnant mice led to intestinal and metabolic perturbations of their pups, suggesting that acesulfame K-induced microbiota dysbiosis could lead to inter-generational effects [24]. Notably, all of these studies were conducted using rodent models and no controlled trials have been performed in humans, warranting further research.

**Aspartame (E951)** was associated with changes to microbiota composition and adverse health impacts in 50% (total n=8 studies) of relevant records retrieved. Low-dose aspartame consumption (~13% ADI) was associated with microbiota changes and higher glycemic index in

rats, though food and water consumption also contrived vs. control animals [29]. The same aspartame dose also induced microbiome dysbiosis in pregnant mice, a phenotype transferred to the offspring [30,31]. However, comparable effects were not observed in humans receiving a similar dose (~14% ADI) in a randomized crossover trial [32]. In another randomized controlled trial with humans receiving a low dose of aspartame (~8% ADI), no effects were observed on glycemic index or microbial diversity, but significant effects were observed on functional gene pathway abundance via shotgun metagenome analysis [33]. Subchronic exposure to NNSs, including aspartame, was associated with higher glycemic index in mice [9], though this study grouped different NNSs during statistical analysis so it is unclear if aspartame also contributed to the observed effects.

**Saccharin (E954)** has exhibited mixed results in reviewed literature, with 14% of studies reporting adverse health effects, 7% positive, and 43% no effect (other studies provided no details or were inconclusive; total n=14 studies). In rodent studies, saccharin altered fecal microbiota and metabolomes, associated with elevated liver inflammation markers [34]; however, even at doses above the ADI, only minor alterations were observed in the plasma metabolome [27]. Short-term exposure (2 wk) failed to elicit any changes in microbiota, metabolome, or glycemic response in mice or one randomized controlled human trial [35], but elicited highly significant changes in the same parameters in another randomized controlled human trial [33]. Subchronic exposure (11 wk) at a high dose (~650x ADI [19]) in mice also induced significant changes to microbiome, metabolome, and glycemic responses [9]. Notably, these adverse phenotypes were recapitulated following fecal transfer to germ-free mice, suggesting a causative role of perturbed microbiome in glycemic response and other observed effects [9,33]. Differences in dose, background diet and health status, as well as statistical procedures could explain some of the inconsistencies in the literature regarding human trials, which will require further analysis and follow-up studies to find consensus regarding the effects of saccharin on gut microbiota and host health.

**Sucralose (E955)** was the most highly studied ingredient of all sweeteners (Figure 2A), with 59% of retrieved records reporting changes to microbiota composition and 47% reporting a negative effect on host health (total n=17 studies). Mice fed a high dose of sucralose (~100x ADI [19]) for 11 weeks exhibited elevated glycemic responses [9], though the authors merged multiple NNS-treated groups for statistical analysis, so it is unclear whether sucralose led to a significant result, and no changes in microbiota were shown. In a randomized controlled trial, adults consuming sucralose (102 mg/day for 2 weeks; below the ADI of 5 mg/kg) demonstrated impaired glucose tolerance and altered microbiota compositions, phenotypes that were transferable to germ-free mice, suggesting a causal relationship [33]. Similar findings have been reported in another randomized trial in adults (48 mg/day for 10 weeks) [36]. Mice receiving sucralose in water for 16 weeks (in doses between 0.001-1% ADI) demonstrated a dose-dependent effect on microbiota composition and alpha diversity, as well as decreased intestinal barrier function, but specific health outcomes were not reported [37]. In a separate study, pregnant mice fed sucralose transferred dysbiotic microbiota to their offspring, which developed increased susceptibility to high-fat-diet-induced hepatic steatosis [38]. Similarly as with acesulfame K [24], this report again suggests that some NSSs can induce inter-generational effects, with long-lasting consequences for intestinal homeostasis.

**Steviol glycosides (E960)** were associated with changes to microbiota composition in 50% (total n=12 studies) of relevant records retrieved, but exhibited mixed results regarding health impacts. Exposure to steviol glycosides was associated with altered microbiota profiles but no effects on glucose tolerance were reported in rodent models [39–41]. No effects were observed on microbiota composition in one *in vitro* study [42]. One other study found that steviol glycoside administration partially protected mice from dextran sodium sulfate-induced colitis [43]. Notably, three studies found that maternal feeding of steviol glycosides could alter gut microbiota, weight gain, glucose tolerance, and memory in their offspring, when fed low [30,31] or high doses [44].

To summarize, results regarding the impact of many sweeteners on gut microbiota and host health are inconsistent in the literature, and may warrant further investigation. Repeated observations of inter-generational effects on host health in particular are an interesting finding. That said, the current evidence does not confirm that such effects are causatively linked to microbiome perturbation and observed changes may be linked to other pathways, warranting further research.

## *Emulsifiers*

Emulsifiers are surface-active agents, which lower the interfacial tension in an emulsion to allow mixing of two immiscible liquids [45]. Codex Alimentarius distinguishes between emulsifiers and emulsifying salts [16], the former having the property of maintaining a homogeneous mixture of an oil phase and a water phase, the latter reorienting proteins in the production of foods to prevent the oil from separating. Due to these properties, emulsifiers are frequently used in food production to prevent sedimentation, flocculation, or separation, resulting in a uniform consistency [46]. Common food emulsifiers include those of natural origin (e.g., lecithin from egg) as well as synthetically produced (e.g., methyl cellulose) [47]. Humans have always been in contact with natural emulsifiers, e.g., through the consumption of eggs, since 20% of the egg yolk consists of phospholipids [45]. Synthetic emulsifiers, however, have been on our plates only for a few decades. This has increased the attention to the effects of emulsifiers on human health [14]. It is important to note that emulsifiers are classified based on their functional properties, not their structural properties, and hence include many chemically diverse compounds with multiple functional properties, including some with beneficial properties for human health, e.g., some dietary fibers (Figure 2B). Thus, some stabilizing agents are also classified as emulsifiers [16], although they do not have surfactant properties (e.g., xanthan gum). In this detailed review, we focus on those with at least some reports of adverse health effects, or with contradictory results, and on those most commonly used in foods: carrageenan, lecithins, mono- and diglycerides of fatty acids, polysorbate, sodium carboxymethyl cellulose (CMC), and xanthan gum.

**Carrageenan (E407)** was associated with changes to microbiota composition in 85% (total n=7 studies) of relevant records retrieved; in 57% of these, adverse health effects were reported, and the remainder reported positive (29%) or inconclusive (14%) effects. Carrageenans have been consumed by humans for centuries, but increasing use in processed foods, lack of absorption during digestion (and thus exposure to the gut microbiota), and reports that carrageenan consumption can induce colitis in animal models have made it a target for comprehensive safety assessments [48]. In rodent models, carrageenan administration alters

bacterial diversity [49], reduces short chain fatty acid (SCFA) production, decreases gut mucosal thickness, and reduce colonization resistance, suggesting that carrageenans do not directly induce inflammation, but create more susceptible conditions for inflammation [50,51]. In an *in vitro* gut model, carrageenans exerted significant impacts on microbiota density, composition, and expression of pro-inflammatory molecules [52]. Conversely, carrageenan supplementation was reported to reduce obesigenic effects in mice fed high-fat diets [53]. The delivery mode of carrageenan (in water or in a food matrix; [54]) and the salt/sucrose content of the diet [55] appear to modulate pro-inflammatory effects. These observations suggest that context matters, and can potentially explain inconsistencies in the literature, as well as limitations for assessing safety in foods [48]. Differences in molecular weight distributions of different carrageenan preparations could also explain some inconsistencies reported in literature [48].

**Lecithins (E322)** were associated with changes to microbiota composition in 3 out of 4 records (75%), but no negative health effects were reported in these studies (i.e., 2 reported positive, 1 no effect, and 1 inconclusive effects). Dietary lecithins are typically complex mixtures of glycerophospholipids, and various purified compounds or mixtures are tested in the literature. In animal models, phosphatidylcholine administration is associated with altered gut microbiota and lipid metabolism profiles [56], protection from colitis [57], protection against inflammation and cognitive impairment [58], and improved insulin resistance [59]. Lecithins also had minimal impact on *in vitro* gut microbiota models [52].

**Mono- and diglycerides of fatty acids (E471)** used as food additives were associated with changes to microbiota composition in 83% (total n=6) of records, but with predominantly positive effects on host health (4 out of 6 studies). One study reported metabolic syndrome, intestinal dysbiosis, and inflammation in mice fed a low-fat diet supplemented with glycerol monolaurate [60], though this treatment was associated with amelioration of obesity [61] and metabolic syndrome in mice fed a high-fat diet [62] and protection from dextran sodium sulfate-induced colitis [63].

**Polysorbates (E430–E436)** were associated with adverse health effects in 92% (total n=13 studies) of records retrieved. In mice, polysorbate 80 (P80) administered *ad libitum* in water induced low-grade inflammation and obesity/metabolic abnormalities, with germ-free mouse experiments confirming a causative link to microbial perturbation; however, food intake was also elevated in the treated mice vs. controls [5]. In mice, P80 administration also elicited sex-dependent effects on gut microbiota and host behavior [64], growth of sulfide-producing bacteria and susceptibility to intestinal inflammation [65], exacerbation of irradiation enteritis [66], and even colorectal cancer in a microbiome-dependent fashion [67,68]. Notably, intestinal dysbiosis induced by P80 administration to pregnant mice led to dysbiosis and colitis susceptibility in their offspring [69]. Similar disruptions to the microbiome and microbial metabolome have been also observed in *in vitro* gut models [52,70,71].

**Sodium carboxymethyl cellulose (CMC; E466, E469)** was associated with microbiota disturbance and with negative health effects in 86% (total n=7) of records. In mice, CMC administered *ad libitum* in water induced low-grade inflammation and obesity/metabolic abnormalities linked to microbial perturbations (as shown with P80) [5]. In mice, CMC was also

associated with sex-dependent effects on gut microbiota and host behavior [64] and microbial-dysbiosis-linked colorectal cancer [67,68]. In germ-free mice colonized with human microbiota, CMC induced more severe colitis symptoms than P80 under controlled conditions [72]. In a randomized controlled feeding trial in humans, CMC induced abnormal microbiota and metabolome profiles, including reduced concentrations of immunoregulatory SCFAs, and increased microbiota encroachment (a marker of gut inflammation) in a subset of patients [6].

**Xanthan gum (E415)** impacts on gut health have been relatively less studied (total n=3 studies), and overall no negative health effects were reported. Xanthan gum, a microbial polysaccharide, is chemically dissimilar from other naturally occurring polysaccharides that have been a part of human diets historically. As a result, microbes with xanthan degradation genes are enriched in the gut microbiota of industrialized populations [73]. Interestingly, this finding reflects changes in food systems due to spread of industrialization and corollary change in human diets that include increasing amounts of food additives [10–12]. Contrary to other food emulsifiers, exposure to xanthan gum appears to be either neutral or even beneficial in animal studies, including increased colonization resistance to pathogens in mice, increased SCFA production [74] and improved metabolic markers in diabetic rats [75]. Accordingly, xanthan gum has no ADI limit and no recognized health risk based on the assessment of the European Food Safety Authority [76].

Unfortunately, many of the animal studies conducted have been poorly controlled and with unreported covariates, e.g., to ensure that water and feed intake were not altered with treatment. The doses and precise compositions of emulsifier supplements (which tend to be complex mixtures, not chemically defined substrates) and mode of delivery are often not adequately reported in literature. This is an important issue to consider, given that for example the mode of delivery (e.g., in water instead of feed) could exacerbate effects of some emulsifiers, as shown with carrageenans [54], as emulsifier solutions would be more accessible to gut microbiota than those in food mixtures. Similarly, dosing aqueous solutions of emulsifiers in *in vitro* studies may not adequately model conditions in the intestine, e.g., in which some compounds may be partially digested, absorbed, and/or adsorbed in complex matrices of food/digestate.

## 3.3. Classification of methodology for microbiome and food additive assessments

The term “microbiome” refers to a complex and holistic entity, consisting of the microbial communities living in a given environment as well as their constituents, functional activities, and interactions [77]. Hence, diverse methods are necessary to study different aspects of microbiomes. Each method has its own strengths and weaknesses and no single method can achieve all possible objectives for comprehensive analysis of microbiomes. In general, methods can be classified as “targeted” and “untargeted” methods. Targeted methods detect specific microbial species or groups, e.g., via selective cultivation or detection of specific DNA markers. Untargeted methods enable detection and differentiation of many microbial species simultaneously, e.g., by DNA sequencing of heterogeneous regions of universal marker genes.

Hence, untargeted methods have become favored for characterizing complex microbial communities, due to their ability to profile mixed microbial communities without *a priori* knowledge of their composition.

The complex nature of microbiomes has led to recent interest in “multi-omics” approaches to study microbiomes, i.e., by integrating multiple different “omics” technologies to profile multiple aspects of microbiome composition and behavior. “Omics” collectively refers to various untargeted, high-throughput methods for biochemical analysis, such as genomics (the original source of the “omics” suffix), metagenomics, metabolomics, (meta)transcriptomics, and (meta)proteomics. This should not be confused with earlier untargeted methods for molecular profiling, which exhibit lower throughput and resolution, or with unrelated methodologies [78]. Strengths and weaknesses of some of the most common methods for microbiome analysis are listed in Table 2.

**Table 2**. Overview of common methodological approaches used for microbiome profiling.

| Method | Description | Strengths | Weaknesses |
|---|---|---|---|
| Marker-gene amplicon sequencing | Next-generation sequencing of “universal” marker genes (e.g., 16S rRNA genes) for taxonomic identification. | Rapid taxonomic profiling, relatively low cost, mature methodology. | Limited taxonomic resolution (genus or species), primer biases can limit coverage, no direct assessment of gene content. |
| Shotgun metagenome sequencing | Next-generation sequencing of all genetic content. Enables both taxonomic profiling and gene annotation. | Cultivation-free genome reconstruction, both taxonomic and functional profiling. | High cost, technological difficulty of analysis, host/non-targeted DNA contamination. |
| Metatranscriptomics | Next-generation sequencing of all RNA content. Enables profiling of transcriptional activity in microbiomes. | Information on gene expression and functional activity of viable fraction of microbiome. | High cost, specialized protocols for sample preservation and RNA purification, host RNA contamination. |
| Metabolomics | Mass spectrometry of all (untargeted) or selected (targeted) metabolites, typically preceded by a separation via chromatography. Enables identification and quantification of metabolites. | Information on diversity and concentrations of a wide range of metabolite classes with various biochemical actions. | Targeted metabolomics relies on *a priori* information, metabolites recovered with untargeted metabolomics depend on choice of analytical methods. A large number of unannotated metabolites. |
| Metaproteomics | Untargeted mass spectrometry of all proteins from microbial systems. Enables simultaneous protein separation, quantification and identification. | Direct analysis of expressed proteins, direct genotype-phenotype links (via matches to specific microbial lineages). | High cost, technological difficulty of analysis/data interpretation. Many of the proteins have not yet been characterized and their functions are unknown. |
| Quantitative PCR (qPCR) | qPCR is based on detection of fluorescence during amplification of the product (e.g., 16S rRNA genes) from microbial systems. | Enables quantification of total bacterial load within a community, and/or target groups/individual taxa, equipment is widely accessible, cost effective. | Time consuming, primer biases can limit coverage, copy number variation of target genes can impact accuracy, provides no data on functional activity. |
| Cultivation-based approaches | Cultivation of viable isolates or microbial communities that grow on plates and/or liquid media. | Enables detection and isolation of living microbes, provides data on metabolic activity, enables experiments, | Majority of microbes cannot be cultured, risk of contamination, requires trained personnel, relies on additional phenotypic, |

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| | | cost effective method. | molecular or biochemical characterization. |

Untargeted characterization of the microbial species/taxa present in a complex community can only be achieved using DNA-sequencing-based methods. Cultivation bias limits the effectiveness of culture-based methods for profiling microbial communities in complex environments, including the human gut (i.e., as the majority of microbes cannot be cultured), though cultivation is still required for phenotyping individual populations [79]. Hence, cultivation-based methods or DNA-based methods which target a specific population (e.g., QPCR; see Table 2) should not be used on their own for evaluation of an intervention (e.g., a food additive) on the gut microbiome. Untargeted DNA sequencing methods are needed to yield information about the overall composition of a community, though these methods are prone to other biases [80]. Marker-gene sequencing, untargeted sequencing of a specific gene target (e.g., 16S rRNA genes) amplified directly from a population, is a relatively low-cost high-throughput method for microbiome profiling and is thus most commonly used in the literature (Figure 3). This method yields information about microbiota composition but no direct measurement of functional gene content, though this can be inferred indirectly by comparison to available reference genomes [81]. Shotgun metagenome sequencing involves sequencing all DNA present in a mixed sample, allowing characterization of both the taxonomic composition of a sample as well as the functional genes present in a community. This method may be relevant in the context of food additive research, e.g., to assess metabolic pathways involved in degradation of a novel substrate [73] or genes linked to health outcomes, e.g., pathogenesis genes. However, this is a significantly more expensive and technically challenging technique, and hence is less widely used in food additive research (Figure 3). Both marker-gene sequencing and shotgun metagenome sequencing are powerful methods and widely used in studies examining the impact of food additives on the gut microbiota. However, these methods on their own are primarily explorative and hypothesis-generating; demonstration of a significant change in gut microbiota composition or gene content is not in itself indicative of an effect on host health, and should not be used for inferring safety issues.

Regardless of the method used, most studies use diversity metrics to assess changes in the microbiome. **Alpha diversity** metrics quantify within-sample diversity, e.g., the number of sequence variants, species, or phylogenetic diversity present in an individual sample, which can then be compared across sample groups. **Beta diversity** metrics quantify the dissimilarity of gut microbiota composition between samples, e.g., based on the number of shared species, phylogenetic similarity, et cetera. These methods are very common in microbiome research, as they reduce complex, high-dimensional microbiome measurements (which can include hundreds or thousands of species detected in a set of samples) into more easily quantifiable and comparable metrics [13]. However, in spite of their utility it is important to consider in the context of food additive research that these metrics cannot imply health risks on their own. These metrics quantify differences in the microbiome, and the magnitude of those differences, but interpretation is more complicated. Changes in alpha diversity (e.g., after feeding a food additive) cannot necessarily imply a positive or negative outcome on its own. In general, higher alpha diversity in the gut microbiome is regarded as beneficial, but it is influenced by a complex

set of factors, including diet, environment, and stressors, and can exhibit regular fluctuations at a low magnitude. Very low alpha diversity in the gut microbiome can indicate a severe disruption, as observed with antibiotic treatment or gastrointestinal disease [82]. However, reported disruption from food additives is a much lower magnitude than those reported for, e.g., antibiotic treatments. Similarly, significant differences in beta diversity only show that a change occurred (e.g., vs. control group), and does not necessarily imply a health risk. The microbiome can be highly responsive to many short-term and long-term dietary inputs, manifesting as changes in beta diversity [83]. Hence, changes in beta diversity due to food additives are not a surprising result, in general reflect relatively small differences (as seen in the literature reviewed), and should not be interpreted on their own as concerning changes.

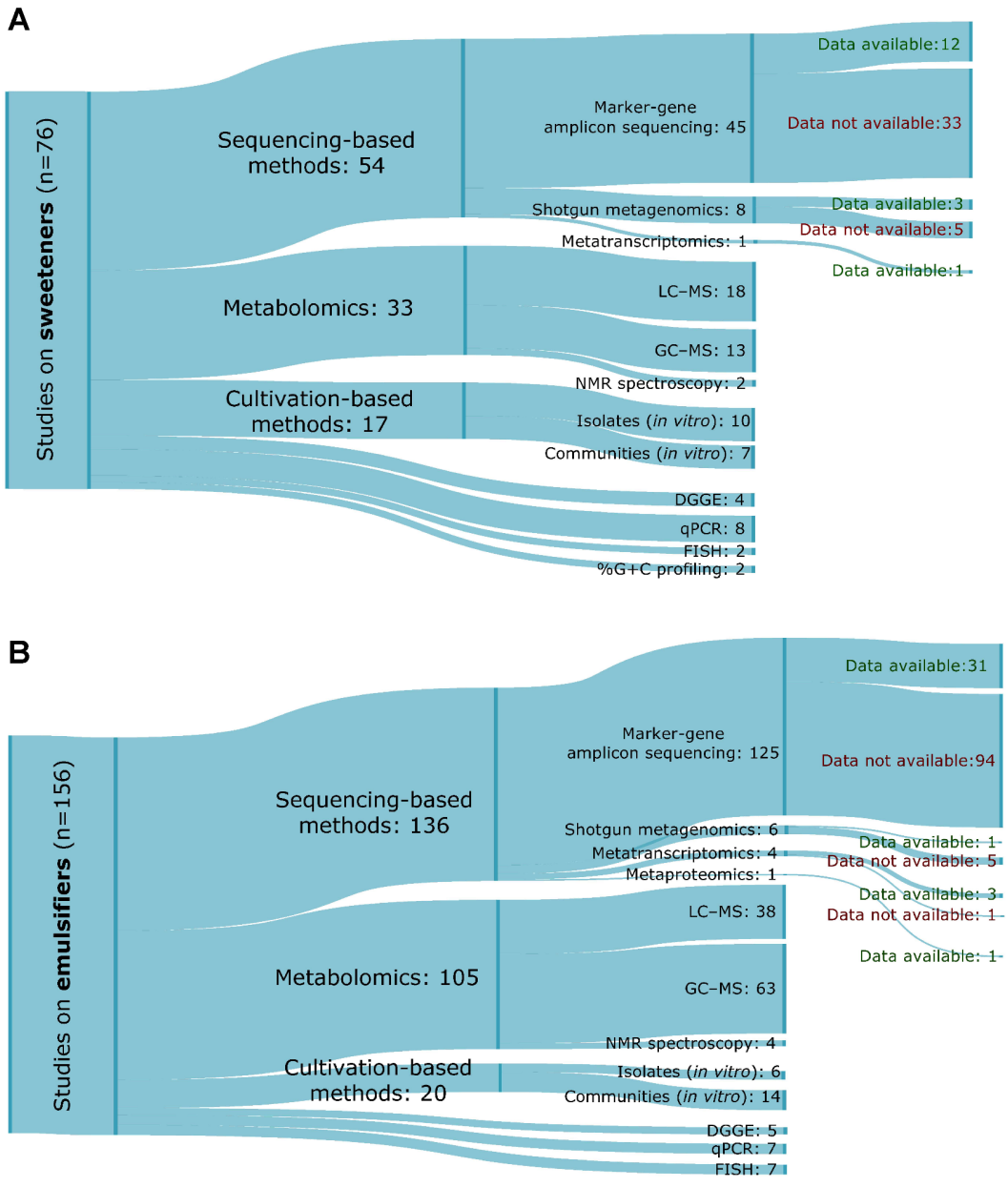

**Figure 3**. Methodological approaches used to study the impacts of food additives (A) sweeteners and (B) emulsifiers on the gut microbiota based on the reviewed literature (n=76 articles for sweeteners, and n=156 for emulsifiers, see Supplementary Data package). Node width is proportional to the numbers indicating the frequency of use of each method in counts. Node totals coloured in green and red indicate the availability of associated data in public data repositories for studies using untargeted sequencing-based methods.

In general, no single method should be considered adequate on its own for evaluating host-microbiome interactions. This also includes the assessment of food additive impacts on human gut health, which essentially hypothesizes that normal host-microbiome interactions are disrupted by a given food additive, either (i) by perturbing the normal composition or activity of the gut microbiota, leading to indirect consequences (e.g., loss of beneficial functions, growth of a pathogen, or changes in immunological or other host pathways) or (ii) via microbial metabolism of food additives, or disruption to normal microbial metabolism. Thus, a combination of methods is needed to assess both microbial composition, as well as microbial metabolism and/or other activities (e.g., via metatranscriptomics, metaproteomics, and metabolomics, see details in Table 2). That said, methods that enable identification and quantification of metabolites, proteins, and other products of microbial metabolism and/or activities are still rarely used in studies of food additives (Figure 3). The most crucial assessment, though, is (iii) what impact this disruption could have on host health, and hence suitable models/methodologies are also necessary to address this question, e.g., glucose tolerance tests [33]. Without these three components, mechanistic proof of food additive disruption cannot be conclusively established.

Disruption of gut microbiota composition should be evaluated via use of an untargeted DNA sequencing method (e.g., marker-gene or shotgun metagenome sequencing) to provide adequate resolution and coverage of diverse microbial lineages. Targeted methods, e.g., cultivation-based approaches and QPCR, could suffice if a precise mechanism is known or evaluated with complementary methods, but otherwise would provide inadequate coverage of microbial diversity in the human gut (Table 2).

## *Model systems and pitfalls*

Food additive assessments have been performed using a wide variety of model systems, as well as human clinical trials. The majority of relevant studies retrieved have used mouse models (45% of sweetener studies, 55% of emulsifier studies) or rats (15% and 9% of studies for sweeteners and emulsifiers, respectively). A small number of studies have used pigs, yet with an apparent trend for more emulsifier studies (15%) using this model compared to sweetener studies (2.5%). Studies in humans accounted for 23% of records retrieved for sweeteners and 17% for emulsifiers. Other mammalian animal models were less common and included primates, cats, dogs, and rabbits.

Animal models have been a crucial component of biomedical and toxicology research over the past decades, and in food additive research they remain critical for pre-clinical safety assessments. However, they also pose significant limitations, particularly for assessing additive-microbiome-host interactions. Rodent models have been criticized for human gut research due to the dramatic differences in gastrointestinal physiology between rodents and

humans [84]. Different microorganisms colonize the gastrointestinal tracts of rodents vs. humans, leading to differences in co-evolutionary host-symbiont interactions, as well as pathogenesis mechanisms, between these hosts [84]. Moreover, laboratory rodents are typically detached from natural environmental conditions and harbor divergent microbiota that can substantially distort the development of the immune system, and thus have limited ability to mirror immune/inflammatory responses and physiology of free-living mammals such as humans [85,86]. Differences in xenobiotic metabolism and physiology are also crucial for interpreting food additive safety assessments in animal models. For example, studies showing that rats fed saccharin developed bladder cancer fail to translate to humans, due to differences in urinary pH and protein content that promote toxic crystal formation only in rats [87]. With this in mind, pigs can provide a powerful alternative non-primate animal model. Indeed, the physiological similarity between humans and pigs in terms of digestion system and metabolic processes can offer a better translational model for microbiome research [88,89]. Together, these findings highlight the importance of randomized controlled clinical trials for confirming food additive safety assessments in humans, except where clear hazards can be shown in pre-clinical models.

Another major consideration for gut microbiota assessments in rodent models is the influence of cage effects and progeny effects on microbiome-linked phenotypes. Mice are coprophagic, and hence cage mates and litter mates tend to share gut microbiota, skewing treatment or genotype effects [90–92]. This effect needs to be carefully considered during experimental design, e.g., to ensure sufficient replication of cages and litters, and (if appropriate) randomization of treatments across litters, to control for this important covariate [13]. In much of the literature on food additives, cage and litter information is underreported, making evaluation and further comparison difficult. For some of these studies, the low sample size suggests that in some cases a single cage may be assigned to a treatment, raising the possibility that observed differences in microbiome composition (and perhaps also observed phenotype) are related to cage effects rather than treatment effects.

Regardless of the host species used, studies should include both sexes for assessments of food additive effects on gut microbiota. Biological sex of the host can shape its gut microbiota, influencing various aspects of host-microbiome interactions [93]. That said, male bias is common in the literature on food additives, with solely male subjects used in 46% of records retrieved for sweeteners and 49% of records for emulsifiers. This is an important issue to consider given that diet, antibiotics, various environmental factors [93], as well as some food additives [64] can impact gut microbiota in a sex-dependent manner, highlighting the importance of considering both host sexes for safety assessments.

A major challenge for *in vitro* models is their capacity to model the human gastrointestinal tract, especially with regard to digestive breakdown of dietary inputs [94–96]. Physiologically relevant digestion models should be used, where relevant, for *in vitro* assessments of food additive effects on gut microbiota. *In vitro* assays should also receive food additive inputs together with model digested foods, as used in models of human digestion, so that food matrix effects can be carefully evaluated to confirm biological relevance. More generally, careful consideration should be given to the digestive fate of food additives in the gastrointestinal tract to determine if *in vitro* fermentation tests are even relevant. For example, aspartame is rapidly hydrolyzed and

absorbed in the small intestine, making it highly unlikely that aspartame or its metabolites are ever exposed to gut microbiota in the colon [97,98]. The digestive fate of food additives must be well understood to allow relevant *in vitro* modeling [52].

Another limitation in some *in vivo* and *in vitro* studies is the selection of a physiologically relevant dose. Some studies of food additives exaggerate potential effects by providing unrealistic dosages, e.g., an order of magnitude above levels expected in an average diet [19]. Exaggerated doses are useful for illustrative purposes, but have limited value for safety assessments. Similarly, many studies on food additives lack ecological reality as researchers predominantly use a cross-sectional study design (i.e., design in which data are collected from many different individuals at a single point in time), with sweeteners and emulsifiers typically administered over short periods of time. A cross-sectional study design is used in 65% of records retrieved for sweeteners and 72% of records for emulsifiers. In contrast, only one third of studies are using longitudinal sampling (i.e., design in which data are collected from the same individuals at multiple time points), yet this experimental design is more relevant for modeling chronic exposure to food additives, which is expected for an average human diet [10–12]. More frequent use of longitudinal study designs is important for more realistic safety assessments.

Finally, despite the increasing recognition of the vital functions that the gut microbiota provide to their animal host, most studies on food additives are correlative, preventing detailed insights into causality. Indeed, correlation between changes in microbiome diversity or composition (e.g., abundance of a key species) and host health measurements (e.g., glucose tolerance) are compelling but not proof of causation. In some studies, antibiotics are used to demonstrate the necessity of an intact microbiome for eliciting a change in host health in an animal model. However, antibiotics can directly exert metabolic changes in the host, independent of the microbiome, and should be avoided. Use of germ-free animals, and particularly the transfer of fecal microbiota from animals treated with a food additive to germ-free animals fed a control diet can be important to demonstrate that the microbiome plays a causative role in any ensuing health phenotype; for example, as shown with NNS impacts on glucose tolerance in germ-free mice [9,33]. However, fecal microbiota transfer to germ-free mice is a “black box” and cannot pinpoint a specific mechanism (or even individual species) driving observed effects. Transfer of defined consortium and gene knockout models (in animal models and/or microbiota) are needed to describe mechanistic interactions, methods that are lacking in the food additive literature to date.

## Other food additives with potential impacts

This report provides a critical review of the evidence and evaluates the potential for different food additives to impact human health via changes in the gut microbiome, with a particular focus on sweeteners and emulsifiers. However, with increasing consumption of processed foods [10–12], many other food additives are now present in human diets. These food additives can be used in food production to improve mouthfeel, enhance taste, or aesthetic properties of dietary products. A small number of recent studies suggest that some of these food additives can also impact host health via gut microbiota-dependent pathways. For example, chronic exposure to commonly used food colorant Allura Red AC promotes susceptibility to colitis via

disruption of gut health in mice [99]. The implication is that while compelling evidence regarding the health effects of some food additives is emerging, the impacts of most food additives that can be found in commonly consumed dietary products have yet to be established.

# 4. Conclusions

Our overall opinion is that investigations of food additive–gut microbiome interactions that impact human health are still at a nascent stage. Overall, the research on the topic is largely preliminary and descriptive, with some compelling results, but more work is needed to make conclusive claims for any of the food additives tested. Many studies here are plagued by common issues with study design, including inadequate sample sizes, poor control of experimental covariates, and unrealistic dosages/model designs. Some recent randomized controlled intervention trials in humans offer compelling evidence that some NNSs and emulsifiers may impact human health via microbiota perturbation [6,33]. In particular, the finding that differences in baseline microbiome lead to variability in responses to NNS feeding [33], opens new avenues for considering how inter-individual variation in gut microbiota influences xenobiotic metabolism and susceptibility to inflammation. However, this remains uncharted territory, and such results should also be considered as preliminary evidence. Moreover, datasets presented in the majority of studies on food additives are not openly available (see Figure 3), restricting reuse of data and preventing a quantitative meta-analysis using standardized methods; a powerful approach to synthesize existing knowledge and to identify consistencies across microbiome studies [100]. As such, the current evidence is correlative, and specific mechanisms of interaction between the food additives and microbiota-induced health outcomes have yet to be established.

We make the following recommendations for interpreting the literature on this topic:

- Demonstrated changes in microbial composition alone are compelling and hypothesis-generating results, but insufficient to demonstrate a specific health risk.
- Longitudinal studies are needed to better evaluate effects of food additives on long-term health. Microbiota often exhibit adaptive responses, i.e., subchronic exposures can gradually promote growth of specific clades capable of metabolizing a given substance or upregulating the metabolic pathways involved, as shown in early *in vitro* studies of cyclamate [23].
- Dosage information needs to be carefully considered, especially for food additives with an ADI recommendation. Some of the inconsistencies in the literature could be due to wide variation in experimental dosage, and some studies test dosages several orders of magnitude above the ADI, rendering the findings physiologically irrelevant [19].
- Results should be confirmed in humans, except where obvious toxic or health effects are reported in animal or *in vitro* models.
- The digestive fate of different sweeteners should be considered when designing and analyzing studies; as some ingredients are rapidly absorbed and/or degraded prior to reaching the large intestine [101], and hence any perceived effects on the microbiome

may be due to covariates (e.g., carrier substances, impacts on water and food consumption, etc).

- Positivity bias could lead to underreporting of null results in the literature. This is a speculative conclusion, but based on the fact that most studies in our critical review showed positive or negative effects of different food additives; null results were considerably more rare.
- The influence of the food matrix on food additive effects is not examined in sufficient detail. Many studies (e.g., in mice) deliver food additives in water, which may lead to differences in activity [54], and may not be physiologically relevant (as most food additives would be consumed as part of a complex mixture).
- Cumulative and additive effects of food additives are insufficiently tested in the literature. Mixtures of different food additives are frequently used in processed foods, raising the possibility that these mixtures could induce amplified or even counteracting effects. The possibility for such interactions deserves more thorough investigation.

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# Appendix 1.

**Table S1.** Food additives and their respective INS E numbers (International Numbering System) used to search published records in PubMed. Ingredients within two functional classes of food additives, *sweeteners* and *emulsifiers* (including emulsifying salts) were used according to terms defined by the international food standards maintained by the World Health Organization and Food and Agriculture Organization of the United Nations (i.e., Codex Alimentarius, document CXG 36-1989 [16]). Every food additive belonging to one of these classes was included in the search terms, resulting in 25 sweeteners and 126 emulsifiers.

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|---|---|---|---|---|---|---|---|---|
| **(A) Sweeteners** used to search published records on PubMed | | | | | | | | |
| **420i** | **E420** | Sorbitol | **957** | **E957** | Thaumatin | **963** | **E963** | Tagatose, D- |
| **421** | **E421** | Mannitol | **958** | **E958** | Glycyrrhizin | **964** | **E964** | Polyglycitol syrup |
| **950** | **E950** | Acesulfame potassium | **959** | **E959** | Neohesperidine dihydrochalcone | **965i** | **E965** | Maltitol |
| **951** | **E951** | Aspartame | **960a** | **E960** | Steviol glycosides from Stevia rebaudiana Bertoni (Steviol glycosides from Stevia) | **966** | **E966** | Lactitol |
| **952** | **E952** | Cyclamates | **960b** | **E960** | Steviol glycosides from fermentation | **967** | **E967** | Xylitol |
| **953** | **E953** | Isomalt (Hydrogenated isomaltulose) | **960c** | **E960** | Enzymatically produced steviol glycosides | **968** | **E968** | Erythritol |
| **954** | **E954** | Saccharins | **960d** | **E960** | Glucosylated steviol glycosides | **969** | **E969** | Advantame |
| **955** | **E955** | Sucralose (Trichlorogalactosucrose) | **961** | **E961** | Neotame | | | |
| **956** | **E956** | Alitame | **962** | **E962** | Aspartame-acesulfame salt | | | |
| **(B) Emulsifiers** used to search published records on PubMed | | | | | | | | |
| **181** | **E181** | Tannic acid (Tannins) | **435** | **E435** | Polyoxyethylene (60) sorbitan monostearate | **487** | **E487** | Sodium laurylsulfate |
| **322i** | **E322** | Lecithin | **436** | **E436** | Polyoxyethylene (65) sorbitan tristearate | **488** | **E488** | Ethoxylated mono- and diglycerides |
| **325** | **E325** | Sodium lactate | **437** | **E437** | Tamarind seed polysaccharide | **489** | **E489** | Methyl glucoside-coconut oil ester |
| **326** | **E326** | Potassium lactate | **440** | **E440** | Pectins | **491** | **E491** | Sorbitan monostearate |
| **327** | **E327** | Calcium lactate | **441** | **E441** | Superglycerinate hydrogenated rapseed oil | **492** | **E492** | Sorbitan tristearate |
| **331** | **E331** | Sodium citrate | **442** | **E442** | Ammonium salts of phosphatidic acid | **493** | **E493** | Sorbitan monolaurate |
| **332i** | **E332** | Potassium dihydrogen citrate | **443** | **E443** | Brominated vegetable oils | **494** | **E494** | Sorbitan monooleate |
| **333iii** | **E333** | Tricalcium citrate | **444** | **E444** | Sucrose acetate isobutyrate | **495** | **E495** | Sorbitan monopalmitate |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **335ii** | **E335** | Sodium tartrates | **445** | **E445** | Glycerol ester of rosin | **500i** | **E500** | Sodium carbonate |
| **336** | **E336** | Potassium tartrates | **446** | **E446** | Succistearin | **541i** | **E541** | Sodium aluminium phosphate, acidic |
| **337** | **E337** | Potassium sodium L(+)-tartrate | **450i** | **E450** | Disodium diphosphate | **542** | **E542** | Bone phosphate |
| **339i** | **E339** | Sodium dihydrogen phosphate | **451i** | **E451** | Pentasodium triphosphate | **900a** | **E900** | Polydimethylsiloxane |
| **340i** | **E340** | Potassium dihydrogen phosphate | **452i** | **E452** | Sodium polyphosphate | **901** | **E901** | Beeswax |
| **34i** | **E341** | Calcium dihydrogen phosphate | **460i** | **E460** | Microcrystalline cellulose (Cellulose gel) | **902** | **E902** | Candelilla wax |
| **343i** | **E343** | Magnesium dihydrogen phosphate | **461** | **E461** | Methyl cellulose | **965** | **E965** | Maltitols |
| **353** | **E353** | Metatartaric acid | **463** | **E463** | Hydroxypropyl cellulose | **966** | **E966** | Lactitol |
| **400** | **E400** | Alginic acid | **464** | **E464** | Hydroxypropyl methyl cellulose | **967** | **E967** | Xylitol |
| **401** | **E401** | Sodium alginate | **465** | **E465** | Methyl ethyl cellulose | **999ii** | **E999** | Quillaia extract type 2 |
| **402** | **E402** | Potassium alginate | **466** | **E466** | Sodium carboxymethyl cellulose (Cellulose gum) | **1000** | **E1000** | Cholic acid |
| **403** | **E403** | Ammonium alginate | **467** | **E467** | Ethyl hydroxyethyl cellulose | **1001** | **E1001** | Choline salts |
| **405** | **E405** | Propylene glycol alginate | **470iii** | **E470b** | Magnesium stearate | **1201** | **E1201** | Polyvinylpyrrolidone |
| **406** | **E406** | Agar | **471** | **E471** | Mono- and di-glycerides of fatty acids | **1400** | **E1400** | Dextrins, roasted starch |
| **407** | **E407** | Carrageenan | **472a** | **E472a** | Acetic and fatty acid esters of glycerol | **1401** | **E1401** | Acid-treated starch |
| **407a** | **E407a** | Processed eucheuma seaweed (PES) | **472b** | **E472b** | Lactic and fatty acid esters of glycerol | **1402** | **E1402** | Alkaline treated starch |
| **410** | **E410** | Carob bean gum | **472c** | **E472c** | Citric and fatty acid esters of glycerol (PFAS) | **1403** | **E1403** | Bleached starch |
| **412** | **E412** | Guar gum | **472d** | **E472d** | Tartaric acid esters of mono- and diglycerides of fatty acids | **1404** | **E1404** | Oxidized starch |
| **413** | **E413** | Tragacanth gum | **472e** | **E472e** | Diacetyltartaric and fatty acid esters of glycerol | **1405** | **E1405** | Starches, enzyme treated |
| **414** | **E414** | Gum arabic (Acacia gum) | **472g** | **E472g** | Succinylated monoglycerides | **1410** | **E1410** | Monostarch phosphate |
| **415** | **E415** | Xanthan gum | **473** | **E473** | Sucrose esters of fatty acids | **1412** | **E1412** | Distarch phosphate |
| **416** | **E416** | Karaya gum | **473a** | **E473a** | Sucrose oligoesters, type I and type II | **1413** | **E1413** | Phosphated distarch phosphate |
| **419** | **E419** | Gum ghatti | **474** | **E474** | Sucroglycerides | **1414** | **E1414** | Acetylated distarch phosphate |

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| **423** | **E423** | Octenyl succinic acid (OSA) modified gum arabic | **475** | **E475** | Polyglycerol esters of fatty acids | **1420** | **E1420** | Starch acetate |
| **425** | **E425** | Konjac flour | **476** | **E476** | Polyglycerol esters of interesterified ricinoleic acid | **1422** | **E1422** | Acetylated distarch adipate |
| **426** | **E426** | Soybean hemicellulose | **477** | **E477** | Propylene glycol esters of fatty acids | **1440** | **E1440** | Hydroxypropyl starch |
| **427** | **E427** | Cassia gum | **478** | **E478** | Lactylated fatty acid esters of glycerol and propylene glycol | **1442** | **E1442** | Hydroxypropyl distarch phosphate |
| **428** | **E428** | Gelatin | **479** | **E479** | Thermally oxidized soya bean oil interacted with mono- and diglycerides of fatty acids | **1450** | **E1450** | Starch sodium octenyl succinate |
| **429** | **E429** | Peptones | **480** | **E480** | Dioctyl sodium sulfosuccinate | **1451** | **E1451** | Acetylated oxidized starch |
| **430** | **E430** | Polyoxyethylene (8) stearate | **481** | **E481** | Sodium lactylate | **1503** | **E1503** | Castor oil |
| **431** | **E431** | Polyoxyethylene (40) stearate | **482** | **E482** | Calcium lactylate | **1505** | **E1505** | Triethyl citrate |
| **432** | **E432** | Polyoxyethylene (20) sorbitan monolaurate | **484** | **E484** | Stearyl citrate | **1518** | **E1518** | Triacetin |
| **433** | **E433** | Polyoxyethylene (80) sorbitan monooleate | **485** | **E485** | Sodium stearoyl fumarate | **1520** | **E1520** | Propylene glycol |
| **434** | **E434** | Polyoxyethylene (40) sorbitan monopalmitate | **486** | **E486** | Calcium stearoyl fumarate | **1521** | **E1521** | Polyethylene glycol |

## Search terms used in PubMed database

**Generic search terms for sweeteners:**

(artificial-sweetener OR artificial-sweeteners OR non-caloric-sweetener OR non-nutritive-sweetener) AND (intestinal-microbi* OR gut-microbi* OR gastrointestinal-microbi* OR intestinal-microflora OR gastrointestinal-microflora OR gut-microflora) AND (2002:2022[pdat]) AND journal article NOT (review OR systematic review)

**Specific search terms for sweeteners:**

(sorbitol OR E420 OR mannitol OR E421 OR acesulfame-potassium OR E950 OR aspartame OR E951 OR cyclamates OR E952 OR isomalt OR E953 OR saccharins OR E954 OR sucralose OR E955 alitame OR E956 OR thaumatin OR E957 OR glycyrrhizin OR E958 OR neohesperidine-dihydrochalcone OR E959 OR steviol-glycosides OR E960 OR neotame OR E961 OR tagatose OR 963 OR maltitol OR E965 OR lactitol OR E966 OR xylitol OR E967 OR erythritol OR E968 OR advantame OR E969) AND (intestinal-microbi* OR gut-microbi* OR gastrointestinal-microbi* OR intestinal-microflora OR gastrointestinal-microflora OR gut-microflora) AND (2002:2022[pdat]) AND journal article NOT (review OR systematic review)

**Generic search terms for emulsifiers:**

(emulsifier OR surfactant) AND (intestinal-microbi* OR gut-microbi* OR gastrointestinal-microbi* OR intestinal-microflora OR gastrointestinal-microflora OR gut-microflora) AND (2002:2022[pdat]) AND journal article NOT (review OR systematic review)

**Specific search terms for emulsifiers:**

(tannic-acid OR E181 OR lecithins OR E322 OR sodium-lactate OR E325 OR calcium-lactate OR E327 OR sodium-citrate OR E331 OR potassium-citrate OR E332 OR calcium-citrate OR E333 OR potassium-sodium-tartrate OR E337 OR sodium-phosphate OR E339 OR potassium-phosphate OR E340 OR metatartaric-acid OR E353 OR alginic-acid OR E400 OR sodium-alginate OR E401 OR potassium-alginate OR E402 OR propylene-glycol-alginate OR E405 OR agar OR E406 OR carrageenan OR E407 OR carob-bean-gum OR E410 OR guar-gum OR E412 OR tragacanth-gum OR E413 OR gum-arabic OR E414 OR xanthan-gum OR E415 OR karaya-gum OR E416 OR konjac-flour OR E425 OR soybean-hemicellulose OR E426 OR gelatin OR E428 OR peptones OR E429 OR polysorbate OR E430 OR E431 OR E432 OR E433 OR E434 OR E435 OR E436 OR tamarind-seed-polysaccharide OR E437 OR pectins OR E440 OR brominated-vegetable-oils OR E443 OR succistearin OR E446 OR diphosphates OR E450 OR triphosphates OR E451 OR polyphosphates OR E452 OR cellulos OR E460 OR methyl-cellulose OR E461 OR hydroxypropyl-cellulose OR E463 OR methyl-ethyl-cellulose OR E465 OR sodium-carboxymethyl-cellulose OR E466 OR magnesium-stearate OR E470b OR lactylated-fatty-acid-esters OR E478 OR dioctyl-sodium-sulfosuccinate OR E480 OR sodium-lactylate OR E481 OR calcium-lactylate OR E482 OR sodium-laurylsulfate OR E487 OR methyl-oil-ester OR E489 OR sorbitan-monooleate OR E494 OR sodium-carbonate OR E500 OR sodium-aluminium-phosphates OR E541 OR polydimethylsiloxane OR E900 OR beeswax OR E901 OR cholic acid OR E1000 OR choline

salts OR E1001 OR polyvinylpyrrolidone OR E1201 OR dextrins OR E1400 OR alkaline-treated-starch OR E1402 OR starches OR E1405 OR E1450 OR distarch-phosphate OR E1412 OR phosphated-distarch-phosphate OR E1413 OR castor-oil OR E1503 OR triethyl-citrate OR E1505 OR propylene-glycol OR E1520 OR polyethylene-glycol OR E1521) AND (intestinal micro* OR gut micro*) AND (intestinal-microbi* OR gut-microbi* OR gastrointestinal-microbi* OR intestinal-microflora OR gastrointestinal-microflora OR gut-microflora) AND (2002:2022[pdat]) AND journal article NOT (review OR systematic review)

# Results from a qualitative meta-analysis

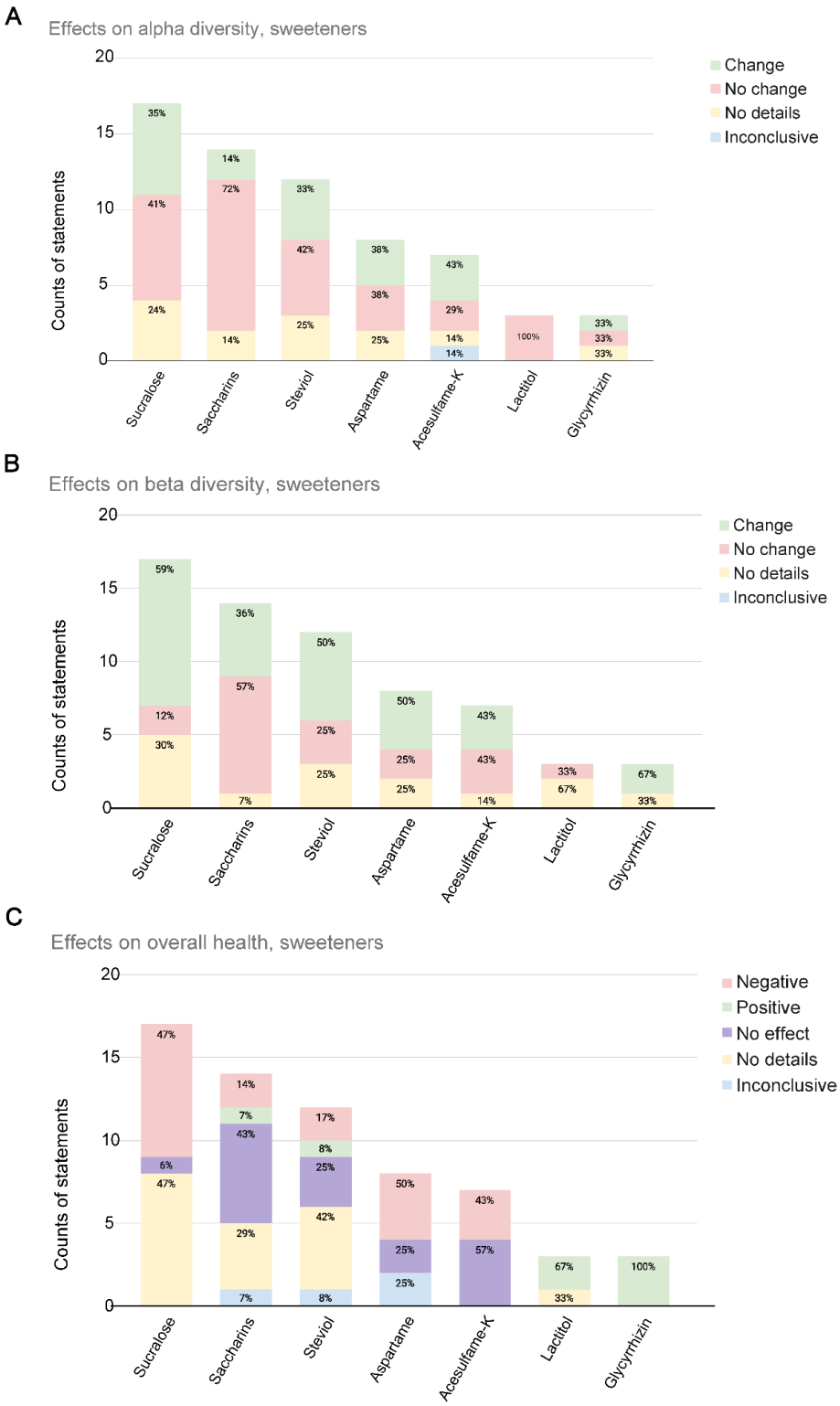


**Figure S1**. Results of a qualitative meta-analysis on the impacts of sweeteners on the gut microbiota (A) alpha diversity, (B) beta diversity, and (C) host health based on study outcomes (i.e., information provided by the original authors). For methods and definitions of the classification terms see *Section 2.4*.

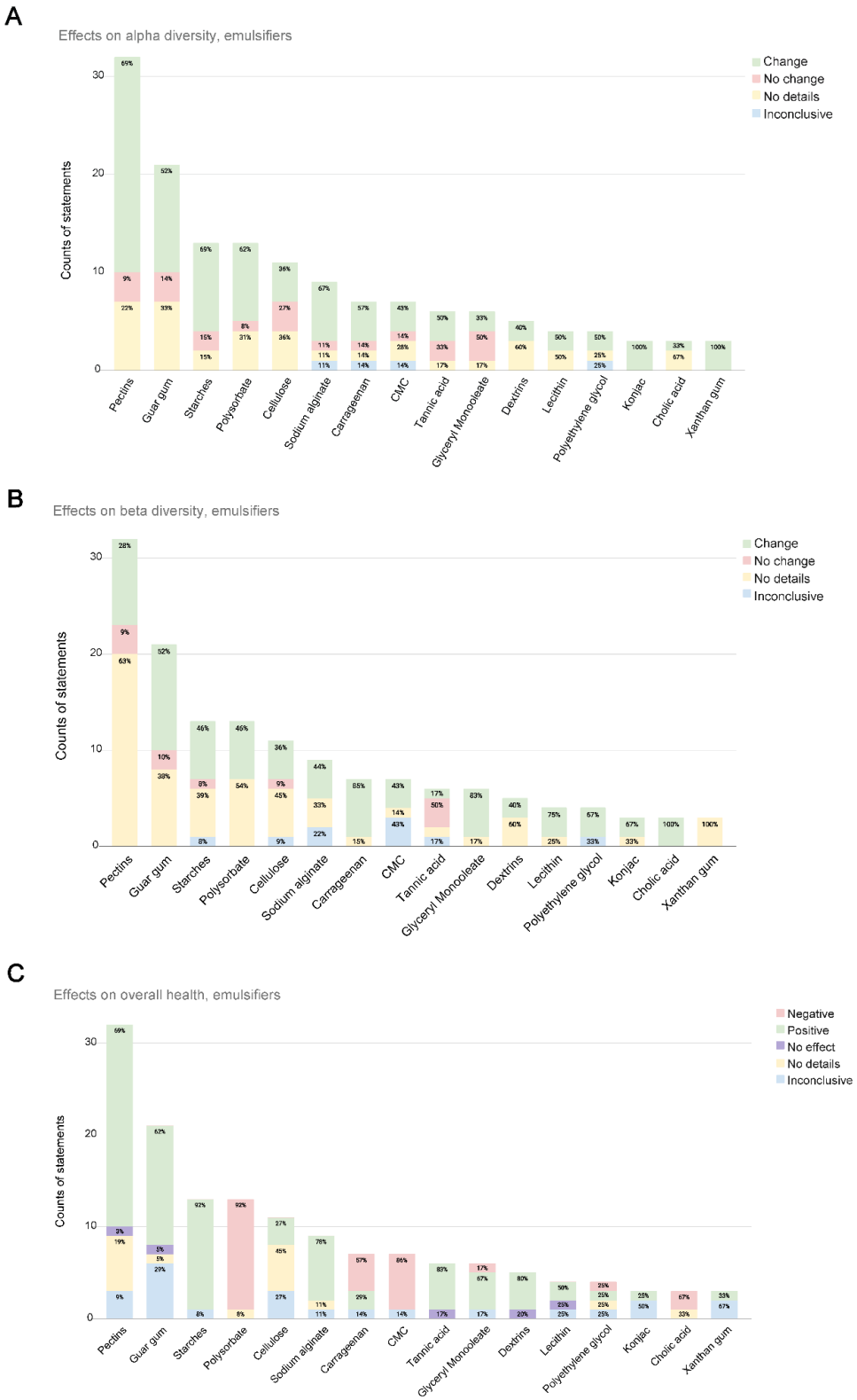


**Figure S2**. Results of a qualitative meta-analysis on the impacts of emulsifiers on the gut microbiota (A) alpha diversity, (B) beta diversity, and (C) host health based on study outcomes (i.e., information provided by the original authors). For methods and definitions of the classification terms see *Section 2.4*.