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TITULO DE LA MEMORIA:

Vegetable smoothies as a source of bioactive compounds potentially modulators of the gut microbiota

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Resumen

Las verduras son esenciales en una dieta saludable por su contenido en micronutrientes, fibra y compuestos bioactivos. El objetivo de esta revisión es investigar si las verduras utilizadas en los batidos se pueden considerar una fuente relevante de compuestos bioactivos, específicamente polifenoles. Así como, parte del objetivo es evaluar si estos polifenoles presentes en las verduras frecuentemente consumidas en los batidos tienen el potencial de modular la microbiota intestinal. Los resultados fueron los siguientes. Los compuestos fenólicos más abundantes en la col rizada fueron derivados de kaempferoles y quercetina, en la espinaca fueron los flavonoles, en el pepino el kaempeferol y la quercetina, la furanocumarina en el apio, la luteolina en la remolacha y en la calabaza, y los ácidos fenólicos en la zanahoria. Todas las verduras estudiadas, excepto por la calabaza y el pepino ya que no se encontraron estudios, tuvieron la capacidad de modular la microbiota actuando como prebióticos y estimulando la producción de ácidos grasos de cadena corta.

Palabras clave: Microbiota intestinal, Polifenoles, Flavonoides, Vegetales

Abstract

Vegetables are very important components of a healthy diet as they are sources of micronutrients, fiber, and bioactive compounds. The aim of this review is to explore whether vegetable smoothies can be regarded as a relevant dietary source of bioactive compounds, particularly polyphenols. Additionally, it aims to assess whether the polyphenols found in the vegetables commonly consumed in these smoothies have the potential to modulate the gut microbiota. The vegetables studied for their frequent use in vegetable smoothies were kale, spinach, celery, cucumber, carrots, beets, and pumpkin. The results showed that the most abundant polyphenols were kaempferol derivatives and quercetin in kale, flavonols in spinach, quercetin and kaempferol in cucumber, furanocoumarins in celery, luteolin in beetroot and pumpkin, and phenolic acids in carrot. All the vegetables studied, except for pumpkin and cucumber for which no evidence has been found, showed to modulate the gut microbiota, exerting a prebiotic effect, and increasing short-chain fatty acid production (SCFA).

Keywords: Gut microbiota, Polyphenols, Flavonoids, Vegetable

1 Introduction

1.1 Balanced diet

A balanced diet is one of the most important contributors to the primary prevention of developing chronic diseases. It is essential for good health. The Mediterranean diet has been proven to be one of the best dietary patterns to follow to maintain or restore good health. It consists of a high intake of vegetables, fruits, nuts, legumes, olive oil, and unprocessed cereals followed by a moderate intake of dairy products and animal protein, prioritizing lean meats such as poultry, white and blue fish, and finally a reduced intake of processed foods and red meat (1). Although all food groups are important, fruits and vegetables are of special importance as they are the main sources of micronutrients, vitamins, and minerals in the diet, as well as non-nutrient components such as bioactive compounds, known for their action as antioxidants which promote good health (2). However, vegetables seem to be the most neglected, as most people don't eat the daily recommended servings (2). A creative way to help reach the daily vegetable serving recommendations is by drinking "green smoothies" or "vegetable smoothies", in which vegetables are blended with fruits and/or other ingredients of choice. Adding vegetables to smoothies boost the smoothie's nutritional value while at the same time making it a more pleasant experience, especially for those who do not enjoy the taste, as in smoothies the taste of vegetables can be hidden. It is important to state that usually, it is preferable to consume fruits and vegetables in their whole state to maintain their original content of vitamins and fiber, but smoothies might be an alternative way to increase vegetable consumption, especially for people whose diets are deficient in these food groups.

1.2 Bioactive compounds

Bioactive compounds can be defined as food components that are not nutrients and can interact with bodily tissues and gut microbes, consequently providing health benefits (3). Foods that have been frequently studied for their bioactive compound content, specifically phenolic compounds, are berries, pomegranates, coffee, red wine, green tea, and cocoa among other fruits and vegetables (4). Most polyphenols are not absorbed throughout the gastrointestinal (GI) tract, reaching the colon where they are metabolized by the gut microbiota (5). Evidence has shown how these compounds are metabolized by the colon releasing metabolites that can modulate the gut microbiota and promote optimal gut health as well as overall health (3).

1.3 Gut microbiota

The gut microbiota is made up of trillions of microorganisms, predominantly bacteria, some of which are beneficial bacteria while others are pathogenic bacteria (6). The state and the composition of intestinal microbiota play a crucial role in health as it is involved in the regulation and optimization of certain mineral balance and absorption like iron, calcium, and magnesium (7). The gut microbiota is also necessary for creating short-chain fatty acids (SCFA) which are essential for producing and maintaining healthy enterocytes, allowing for a healthy gut barrier. The microorganisms in the gut microbiota produce mucins that protect the GI tract from pathogens and toxic substances (7). The specific microorganisms that constitute the gut microbiota are also responsible for modulating the immune system and participate in the production of certain vitamins such as the B and the K vitamins (7). Thus, incentivizing people to take care of their gut health is beneficial for intestinal homeostasis, adequate nutrient absorption, and positive immune system modulation.

1.4 Modulation of the gut microbiota

The gut microbiota is shaped by certain factors that can alter its composition, quantitatively and/or qualitatively, beneficially, or negatively. The microbiota is influenced by environmental exposures, medication, specifically antibiotics, stress, inflammation, physical activity, and **diet** (3). So, lifestyle changes such as diet modification are one of the most accessible and important ways for people to take care of their gut microbiota. There is a bi-directional relationship between gut microbiota composition and phenolic compound metabolism (8). A healthy gut microbiota allows for optimal digestion and release of bioactive compound metabolites. These metabolites are the components that have a physiological effect on the body (3). In return, the released metabolites act as prebiotics which helps create more SCFA which promotes the growth of beneficial bacteria (3). Studies have shown how healthy diets, like the Mediterranean diet, have been associated with higher gut microbiota diversity, providing more beneficial health effects (3). On the contrary, a Western dietary pattern, high in saturated fats and sugar and low in fiber, has been associated with lower gut microbiota diversity (3). Some of the negative consequences of following an unhealthy diet, such as the Western diet, is a gut microbiota high in pathogenic bacteria which can stimulate, in worst cases, the growth of neoplasms in the GI tract due to the production of mutagens and carcinogens as well as a possible increase in the risk of developing mental health and metabolic disorders (7).

The aim of this review is to explore whether vegetable smoothies can be regarded as a relevant dietary source of bioactive compounds, particularly polyphenols. Additionally, it aims to assess whether the polyphenols found in the vegetables commonly consumed in these smoothies have the potential to modulate the gut microbiota.

2 Materials and methods

Research for this review was conducted in **three different steps**.

The **first step** was to search for smoothie recipes. The search terms were "green smoothie recipes" or "vegetable smoothie recipes", on Google and social media platforms. This search was made to see which vegetables came up the most in recipes, determining which vegetables were used more frequently among vegetable smoothie consumers. After browsing through social media platforms such as Instagram and Pinterest and performing a Google search, the ingredients that came up the most were: celery, cucumber, leafy green vegetables such as spinach and kale, carrots, beets, and pumpkin.

Next, the **second step** in the research process was to search for bioactive compound content in the vegetables chosen in the previous step. For this, the food composition databases: Base de Datos Española de Composición de Alimentos (BEDCA), Food data central database from the United States Department of Agriculture (USDA), Food Composition (FoodEx 2 description) from the European Food Safety Authority (EFSA) and Phenol-Explorer (PE) were used to determine bioactive compound composition. In addition, some PubMed articles found in the third step of the research were also used to complement the food composition information (9,10). Afterward, to correctly categorize the bioactive compounds into their respective compound group or family class, other PubMed articles on general information about bioactive compounds were used as guidance (2,11,12).

Finally, the **third step** of the research process was an advanced search in three different bibliographic databases: PubMed, Scopus, and Web of Science using the same Boolean terms and filters. The selected keywords were: Green smoothie OR Vegetable OR Vegetables OR Leafy greens OR Smoothie AND Bioactive compounds OR Phenolic compounds OR Antioxidants OR Prebiotics OR Fiber AND Gut microbiota OR Gut microbiome. Six filters were applied to the results: Text availability: Free full text, Full text; Article type: Article, Review Article; Publication date: 5 years (2019-2023); Species: Humans; and Language: English. When all the sources were listed and the repeated sources were eliminated, the following inclusion and exclusion criteria were applied to select the most relevant sources based on the title and abstract reading.

The **Inclusion criteria** applied include:

- Articles about bioactive compounds and gut microbiota or gut health interaction
- Articles about polyphenols or specific polyphenols and their role in the microbiota

The **exclusion criteria** applied include:

- Articles focused specifically on bioactive compounds in foods that were not one of the smoothie ingredients (kale, spinach, celery, cucumber, beets, carrots, and pumpkin). For example, Polyphenols in flaxseeds or raisins.
- Articles focusing on probiotics.
- Articles focused on a very specific life stage, such as children or pregnant women or lactating women, or people who have specific diseases (Alzheimer's, Non-alcoholic fatty liver disease, depression, covid, etc.).

After applying the inclusion and exclusion criteria, additional sources were excluded based on their titles or after reviewing the abstracts. The results of the advanced search mainly consisted of articles providing general information about the impact of bioactive compounds on gut microbiota. Some of these articles were valuable in gaining a deeper understanding of the mechanisms by which diet can influence the gut microbiota, aligning with the inclusion criteria of "articles about bioactive compounds and gut microbiota." A few of these articles included helpful cross-references to studies conducted on the modulation of gut microbiota by the vegetables in question, primarily involving *in vivo* studies or *in vitro* experiments. While these resources proved beneficial for this review, they were limited in number. Unfortunately, no studies were found specifically focusing on smoothies incorporating these vegetables as ingredients. Consequently, specific separate searches were conducted on PubMed for each individual vegetable, including "Bioactive compounds and cucumber (Cucumis Sativus L.)", "Bioactive compounds and celery (Apium graveolens L.)", "Pumpkin (Cucurbita Maxima D.) and polyphenols", "Spinach and polyphenols", and "Kale and gut microbiota." The publication date filter applied to these searches was set to a 10-year period (2013-2023) to allow for a wider margin, considering the limited information available on this research topic. The results yielded articles and studies related to the seven selected vegetables and their polyphenol content or their impact on the gut microbiota. Out of these, eleven were included in this review. The methodology employed in creating this research paper is illustrated in **Figure 1.**

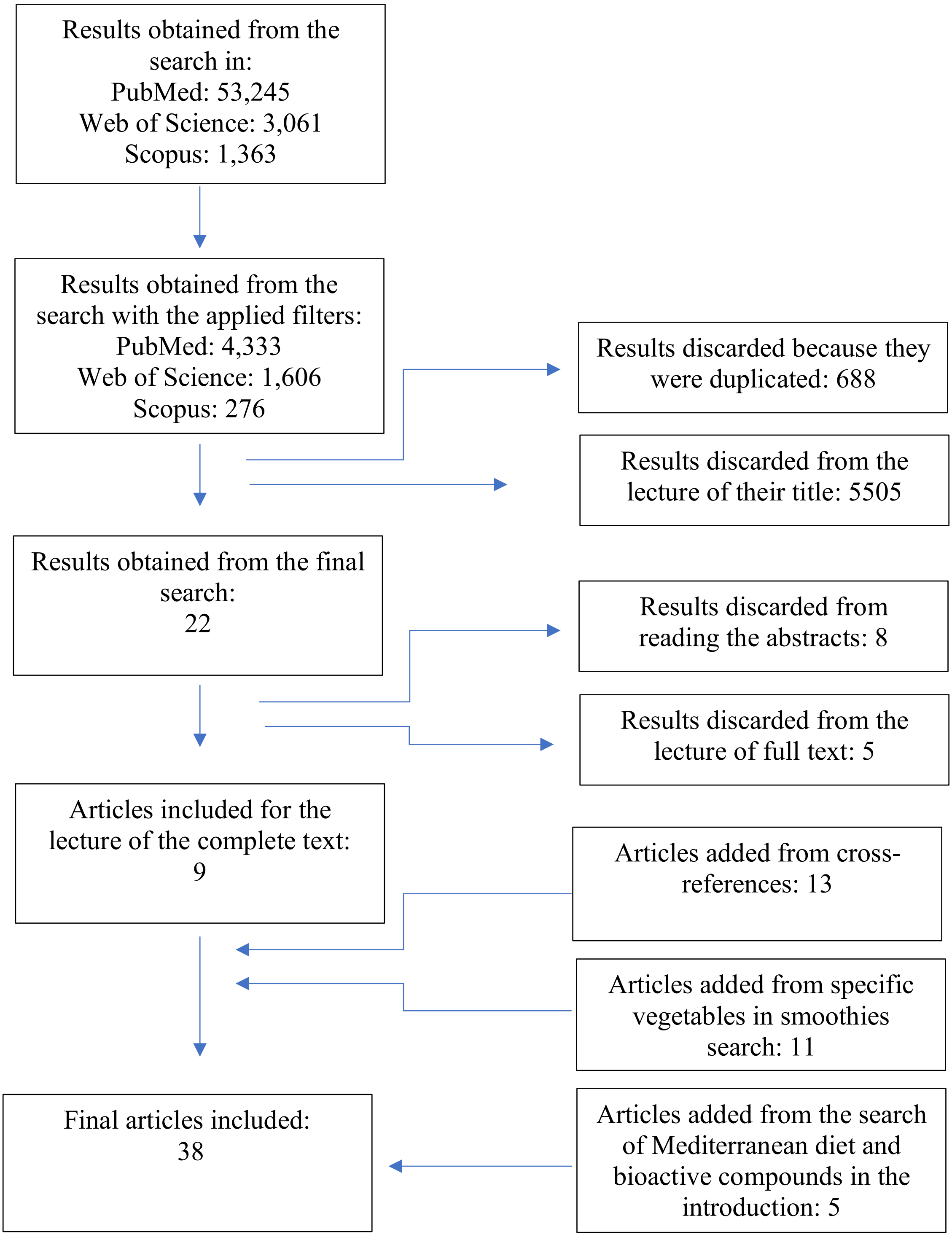

Figure 1. PRISMA flow diagram.

3 Results

Each vegetable has a unique composition profile consisting of nutrients including vitamins and minerals, fiber, pigments, and polyphenols, among others. Vitamins, minerals, and bioactive compounds in vegetables are associated with health-promoting effects (13). Due to the large diversity of vegetables and thus the variety of components comprising this food group, it is important to eat a variety of vegetables. By doing this, people can obtain the benefits from the different nutrients and bioactive compounds that these contain. Bioactive compounds found in plant-based foods are very interesting components as they provide no nutritional value but have been attributed to improving consumers' health by reducing the risk of developing chronic diseases and providing antioxidant activity (7). But more research is necessary to truly understand their mechanism of action to better understand how they exert health benefits (2). Bioactive compounds can be categorized into different compound groups and subgroups (family class). So far, phenolic compounds and carotenoids have been one of the most studied bioactive compounds regarding human health (2). Information on carotenoid content in these vegetables is included in this review, as it is a bioactive compound present along with phenolic compounds. However, the focus of this review is on the polyphenols in these vegetables.

Table 1 shows the bioactive compound content in kale, spinach, cucumber, celery, beetroot, carrot, and pumpkin, obtained from food composition databases (BEDCA, USDA, EFSA, and Phenol-Explorer).

Table 1. Bioactive compounds in vegetables frequently used in smoothies.

Vegetable	Bioactive compound Group	Family Class	Compound Name	Concentration per 100g
Kale *Brassica oleracea* var *Sabellica* L.	Pigment		Chlorophyll	-
	Carotenoids (9)		Beta-carotene	2870 µg (USDA)
			Lutein	6260 µg (USDA)
			Zeaxanthin	
	Phenolic compounds	Flavonols	Kaempferol derivatives	26.74 mg (PE)
			Quercetin	7.71 mg (PE)
		Other flavonoids		(10)
		Tannins		(10)
Spinach *Spinacia oleracea* L.	Pigment		Chlorophyll	(10)
	Carotenoids (9)		Beta-carotene	3400 µg (USDA)
			Lutein	5830 µg (USDA)
			Zeaxanthin	191 µg (USDA)
	Phenolic compounds	Flavonols		119.27 mg (PE)

Table 1. Bioactive compounds in vegetables frequently used in smoothies (continued).

Cucumber *Cucumis sativus* L.	Pigment		Chlorophyll	(10)
	Carotenoids		Beta-carotene Retinol	2 µg (BEDCA)
	Phenolic compounds	Lignans	Lariciresinol	0.05 mg (PE)
			Pinoresinol	8.00×10^{-04} mg (PE)
			Secoisolariciresinol	0.01 mg (PE)
		Flavones	Apigenin	5.00×10^{-04} mg (PE)
			Luteolin	1.5×10^{-03} mg (PE)
		Flavonols	Kaempferol	0.06 mg (PE)
			Quercetin	0.04 mg (PE)
Celery *Apium graveolens* L.	Carotenoids		Retinol Other carotenoids	95 µg (BEDCA)
	Phenolic compound	Coumarin	Furanocoumarin derivatives	2.59 mg (PE)
Beetroot *Beta vulgaris* L.	Pigment		Betacyanin	(10)
	Phenolic compounds	Flavones	Luteolin	0.37 mg (PE)
		Flavonols	Quercetin	0.13 mg (PE)
		Lignans	Secoisolariciresinol	7.08×10^{-03} (PE)
Carrots *Daucus Carota* L.	Carotenoids		Beta-carotene Lycopene	1346 µg (BEDCA)
	Phenolic compounds	Phenolic acids	Hydroxybenzoic acids	0.05 mg (PE)
			Hydroxycinnamic acid derivatives	20 mg (PE)
Pumpkin *Cucurbita Maxima D.*	Carotenoids		Lutein Zeaxanthin Lycopene Beta-carotene	34 µg (BEDCA)
	Phenolic compounds	Flavones	Luteolin	1.63 mg (PE)
		Lignans	Matairesinol	2.5×10^{-05} mg (PE)
			Lariciresinol	0.01 mg (PE)
			Pinoresinol	4.00×10^{-4} mg (PE)
			Secoisolariciresinol	0.10 mg (PE)

USDA: Food data central database from the United States Department of Agriculture. PE: Phenol-Explorer. BEDCA: Base de Datos Española de Composición de Alimentos.

Kale is rich in carotenoids and the most abundant are lutein and zeaxanthin (6260 µg per 100g). The most abundant phenolic compounds in kale are kaempferol derivatives, belonging to the family class flavones (26.74 mg per 100 g). Spinach is also rich in carotenoids, lutein being the most abundant (5830 µg per 100 g), and phenolic compounds belonging to the family class flavonols (119.27 mg per 100 g). Quercetin and Kaempferol, both flavanols, are the most abundant phenolic compounds in cucumber (0.04 mg per 100 g and 0.06 mg per 100g, respectively). Celery is the only vegetable out of the seven containing polyphenols from the family class coumarins, containing furanocoumarin derivatives (2.59 mg per 100g). The phenolic compounds in beetroot include flavones, flavonols, and lignans of which the flavone luteolin has the highest concentration (0.37 mg per 100g). Carrots are known for their carotenoid content. They are rich in beta-carotene and lycopene with a total amount of 1346 µg per 100g of carrots. Carrots contain hydroxybenzoic acids (0.05 mg per 100g), and hydroxycinnamic acids (20 mg per 100g), both being phenolic acids. Pumpkin contains a variety of carotenoids including lutein, zeaxanthin, lycopene, and beta-carotene with a total concentration of (34 µg per 100g). The most abundant phenolic compound in pumpkin is luteolin, from the family class flavones, with a concentration of (1.63 mg per 100g).

Table 2 summarizes the studies found on the polyphenols in the selected vegetables used for smoothies and their effect on gut microbiota. Since no studies were found of smoothies, the information found was separated by the vegetables comprising it. Five *in vivo* studies and one *in vitro* study were found for kale (*Brassica oleracea var Sabellica L.),* spinach (*Spinacia oleracea L.*), celery (*Apium graveolens L.*)*,* carrot (*Daucus Carota L.),* and beetroot (*Beta vulgaris L.*), yet no studies, were found on the effect of polyphenols in cucumber (*Cucumis sativus L*) or the polyphenols in pumpkin (*Cucurbita Maxima D.*) on the gut microbiota. The consumption of kale in an *in vivo* study on obese mice with a high-fat diet (HFD), showed to have a prebiotic effect on their gut microbiota which was attributed to the flavonoid's quercetin and kaempferol in kale (14). An *in vivo* study tested the effect of spinach polyphenols (lignans, flavanols, and phenolic acids) was conducted on two groups of rats, supplemented with spinach. One group of rats was fed a normal diet and the other was fed an HFD. The results observed were a prebiotic effect on both groups. But there was a difference in both groups regarding SCFA production, as the rats with a normal diet increased SCFA production, while the rats with an HFD decreased, despite both groups eating spinach (15). Celery in the form of fresh juice and fermented juice was used to test the effect of the flavonoids luteolin and apigenin on gut microbiota. This experiment was conducted *in vivo* on mice fed with an HFD (16). Both celery juice and fermented celery juice had a prebiotic effect, but the changes were of higher significance in the fermented celery juice, associated with the additional presence of probiotics. An *in vivo* study on humans consuming beetroot juice showed how polyphenols identified in beetroot, quercetin, chlorogenic acid, and ferulic acid influence the gut microbiota by increasing total SCFA production (17). But the results showed no significant change in gut microbiota composition except for an increase in *Akkermansia muciphunila* and *Bacteroides fragilis*. Carrot polyphenol's effect on modulating the gut microbiota was tested in an *in vivo* study on mice by analyzing their feces (18). Results showed a prebiotic effect, increasing SCFA-producing bacteria such as *Lactobacillus* resulting in increased SCFA production, specifically acetic and butyric acids. The same carrot polyphenols were used in an *in vitro* experiment on human feces which also modulated the gut microbiota by increasing *Bacteroidetes* and decreasing *Proteobacteria* and *Firmicute to Bacteroidetes* ratio as well as increasing SCFA production (18).

Table 2. Phenolic compounds in vegetables used in smoothies and their effect on gut microbiota.

Vegetable	Phenolic compound	Source	Type of Study	Duration	Gut microbiota composition	Effect on gut microbiota	Reference
Kale	Flavonoids (Quercetin & Kaempferol)	Kale turned into powder	*In vivo* High-fat diet-fed obese mice with inflammation & dysbiosis	12 weeks	↑*Bacteroidetes, Bacteroidia* ↓ *Firmicutes, Flavobacteria, Bacilli* ↓*Firmicute to Bacteroidetes ratio*	Prebiotic effect	(14)
Spinach	Flavonoids (Patuletin, spinacetin) Phenolic acids (o-coumaric, ferulic, and p-coumaric) Lignans (Lariciresinol, secoisolariciresinol, pinoresinol)	High-fat diet + spinach	*In vivo* Rats	7 weeks	↑ *Bifidobacterium* ↓ *Lactobacillus, Bifidobacterium, Clostridia, Bacteroides*	↓Total SCFA ↑ Acetate	(15)
		Normal diet + spinach			↑*Bifidobacterium*	↓Acetate ↑Propionate	
Cucumber	-	-		-	-	-	-
Celery	Flavonoids (Luteolin & apigenin)	Celery juice Fermented celery juice	*In vivo* High-fat-fed diet mice	13 weeks	↑ *Lactobacillus, Ruminococcaceae, faecalibaculum, blautia* ↓ *firmicute/ Bacteroidete ratio, Alloprevotella, Oscillibacter, Helicobacter, Allobaculum*	Prebiotic effect	(16)
Beetroot	Chlorogenic acid, Ferulic acid & quercetin	Beetroot juice	*In vivo* Healthy Humans	2 weeks	No significant impact in phyla or class level ↓ *Bacteroidiales* ↑ *Akkermansia muciniphula, Bacteroides fragilis*	↑ Total SCFA production ↑Butyric & isobutyric acid	(17)
Carrot	Not specified	Carrot dietary fiber	*In vivo* Mice feces	2 weeks	↑*Lactobacillus rhamnosus, Bacteroides* ↓*Firmicutes, Clostridiales, Ruminococcus, Coprococcus, Oscillospira, dehalobacterium*	Prebiotic effect ↑SCFA production ↑acetic & butyric acid	(18)
			In vitro Human Fecal sample	12 hours	↑ *Bacteroidetes* ↓*Proteobacteria, firmicute/ Bacteroidetes ratio*	Prebiotic effect ↑SCFA production	
Pumpkin	-	-	-	-	-	-	-

SCFA: Short-chain fatty acids.

Studies demonstrating the effect of polyphenols from the selected vegetables on modulation of the gut microbiota used frequently in smoothies are scarce thus, **Table 3** provides information from different studies testing the effect of polyphenol consumption, from different food sources and their ability to modulate the gut microbiota. These food sources contain polyphenols in common with vegetables in **Table 2** allowing to further see how the phenolic compounds from vegetables used for smoothies could affect the gut microbiota. Four studies were found about the effect of flavonoids found in cranberry extract, green tea, and Aronia berry on the gut microbiota. Both studies on cranberry extract and one study on green tea showed to modulate gut microbiota composition having a prebiotic effect while the study on *Aronia melanocarpa* did not modulate gut microbiota composition. But *Aronia melanocarpa* did influence plasma serum inflammatory markers having an anti-inflammatory effect (19). An anti-inflammatory effect was also exerted by the cranberry extract (20,21). Two different studies were found studying the effects of flavonols on gut microbiota. In an *in vivo* study on diet-induced obese rats, these rats' diets were supplemented with a quercetin of 98% purity (22). Quercetin supplementation resulted in the modulation of the gut microbiota, exerting a prebiotic effect, lessening gut dysbiosis, and increasing the production of SCFA (22). Flavonols found in cocoa also showed to have a prebiotic effect on gut microbiota (23). *In vivo,* experiments in humans to test the effect of phenolic acids on gut microbiota were done by administering phenolic acids from different sources such as red wine, dealcoholized red wine, mango pulp, and coffee. Red wine and dealcoholized red wine both had a prebiotic effect on the gut microbiota (24). On the other hand, the *in vivo* study using mango pulp did not have a prebiotic effect on the gut microbiota of the participants, but it did have an anti-inflammatory effect on the gut and enhanced the production of SCFA by the microbiota (25). Three different coffee samples (Nescafé green blend, Nescafé gold blend, and Nescafé original blend) were used as a source of phenolic acids, specifically chlorogenic acid. Out of the three samples, Nescafé green blend had the highest amount of chlorogenic acid (26). All three blends had a prebiotic effect, but the green coffee blend had the most significant impact. Another *in vitro* study using coffee as the source of phenolic acids showed to have a dose-dependent prebiotic effect on the gut microbiota (27). An *in vitro* study utilizing pomegranate juice and pomegranate extract containing ellagitannins also showed to have a selective prebiotic effect on gut microbiota, indicating that tannins can also modulate gut microbiota (28).

Table 3. Phenolic compounds found in other food sources, and their effect on gut microbiota.

Phenolic compound	Source	Type of Study	Duration	Gut microbiota composition	Effect on gut microbiota	References
Flavonoid derivatives	Cranberry extract	*In vivo* diet-induced obese mice	8 weeks	↑ *Akkermansia* ↓*Firmicutes/Bacteroidetes ratio*	Prebiotic effect Anti-inflammatory Inhibit gut barrier dysfunction.	(20,21)
		In vivo healthy humans	2 weeks			
	Green tea	*In vivo* healthy humans	10 days	↑ *Bifidobacteria*	Prebiotic effect	(29)
	Aronia berry *(Aronia melanocarpa)*	*In vivo* Induced colitis rats	2 weeks	-	Anti-inflammatory effect ↓ Pro-inflammatory cytokines	(19)
Flavonols	Quercetin (>98% purity)	*In vivo* diet-induced obese rats	6 weeks	↑ *Akkermansia Municiphila* ↓*Firmicutes/Bacteroidetes ratio, Erysipelotrichaceae, Bacillus, Eubacterium cylindroides, Bilophila wadsworthiacylindroies*	Lessened gut dysbiosis Prebiotic effect ↑SCFAs	(22)
	Cocoa	*In vivo* Healthy humans	8 weeks	↑*Lactobacillus, Bifidobacterium spp, Enterococcus spp, E. rectale–C.*	Prebiotic effect	(23)
Flavones	Luteolin	In vivo Mice with UC	2 weeks	↑*Bacteroidetes* ↓*Firmicutes, Proteobacteria*	Prebiotic effect	(30)
Lignans	-	-	-	-	-	-

SCFA: Short-Chain Fatty Acids; UC: Ulcerative Colitis; IBD: Irritable Bowel Disease.

Table 3. Phenolic compounds found in other food sources, and their effect on gut microbiota (continued).

Phenolic acids	Red wine	*In vivo* healthy men (Randomized crossover, controlled intervention study)	2.5 months	↑ *Enterococcus, Prevotella, Bacteroides, Bifidobacterium, Bacteroides uniformi, Eggerthella lenta, Blautia coccoides–Eubacterium rectale*	Prebiotic effect	(24)
	Dealcoholized red wine			↑*Fusobacteria* ↓*Bacteroidetes Firmicutes*	Prebiotic effect	
	Mango pulp	*In vivo* Humans with IBD	8 weeks	↑Lactobacillus spp	Anti-inflammatory ↓ Pro-inflammatory cytokines ↑SCFAs (butyrate)	(25)
	Coffee (3 samples)	*In vitro*	24 hours	↑*Bifidobacterium, Bacteroidetes* ↑ *C. coccoides-E. rectale*	Prebiotic effect	(26)
	Coffee	*In vitro*	24 hours	↑*Bacteroides, Prevotella, Porphyromonas*	Prebiotic effect (Dose-dependent)	(27)
Ellagitannins (Tannins)	Pomegranate juice and extract	*In vitro*	6 days	↑ *Lactobacillus, Bifidobacterium* ↓ *B. Fragilis group, Clostridia, Enterobacteriaceae,*	Prebiotic effect	(28)

SCFA: Short-Chain Fatty Acids; UC: Ulcerative Colitis; IBD: Irritable Bowel Disease.

4 <u>Discussion</u>

4.1 Bioactive compounds in the vegetables used in smoothies.

Diet is one of the key factors in gut microbiota regulation. Healthy and balanced diets abundant in plant-based foods, and rich in phenolic compounds, promote higher microbial diversity and positively modulate the gut microbiota while on the other hand, unhealthy diets high in saturated fats, sugars and low in fiber can negatively modulate the gut microbiota decreasing bacteria diversity and promoting the growth of pathogens (31). Vegetable smoothie recipes can vary by combining different vegetables. This allows for higher vegetable intake, higher diversity of bioactive compounds in the diet, and higher gut microbiota diversity.

4.1.1 Kale

According to the food composition database Phenol-Explorer, kale is a source of flavonols, kaempferol derivatives, quercetin, other unspecified flavonoids, and tannins. Kale is considered an important source of quercetin and kaempferol exerting beneficial health effects (14). In a study where a new snack was being developed consisting of celery root enriched with vegetable juices, including celery stalk juice, onion juice, and kale juice, to see how these vegetable juices enriched the snack with polyphenols, bioactive compound content was identified using an ACQUITY UPLC-PDA-MS/MS method. After adding the kale juice, the phenolic content changed as kale juice enriched the sample with protocatechuic acid, quercetin derivatives, and kaempferol derivatives, representing phenolic compounds in kale (32).

4.1.2 Spinach

Spinach is a leafy green vegetable and a source of a variety phenolic compounds, which have been associated with anti-inflammatory, antioxidant, and anti-obesity properties due to their bioactive compound content (15). In an *in vivo* study conducted on rats, an important number of polyphenols in spinach were identified including flavonoids (approximately 100-300 mg per 100g) patuletin, spinacetin, spinatoside, and jacedin, and phenolic acids (approximately 40-125mg per 100g) such as o-coumaric, ferulic, and p-coumaric. The Lignans lariciresinol, secoisilariciresinol and pinoresinol were also notable in spinach composition (15). On the other hand, according to the database Phenol Explorer, the phenolic compounds identified in spinach were only flavonols such as Spinacetin 3-O-glucoside, jaceidin 4’-O-glucuronide, patuletin 3-O-glucoside amongst other types of flavanols.

4.1.3 Cucumber

Cucumber contains a diverse number of phenolic compounds. The polyphenols in cucumber include flavonoids, flavones, more specifically luteolin and apigenin, as well as kaempferol and quercetin, from the family class flavonols, and lignans according to the Phenol-Explorer database. An observation from phenolic compound content in the selected vegetables in **Table 1** is that flavones, flavonols, and lignan concentrations in cucumber are lower compared to the concentration of the same compounds in the other vegetables used in smoothies, except for pumpkin which also has very low concentrations of phenolic compounds compared to the rest. Cucumber consumption has been associated with anti-diabetic, antioxidant, antimicrobial, and hypolipidemic properties, beneficial to human health, and is used actively in Indian and Chinese medicine (33). The plant leaves of cucumbers are sources of flavonoids such as apigenin, luteolin, and phenolic acid, hydroxycinnamic acid (33). But it is not habitual to eat cucumber leaves so even though phenolic compounds are present in the leaves, if the leaves are not consumed then this becomes irrelevant, or consuming cucumber leaves could be contemplated. No studies were found on the effect of cucumber bioactive compounds or more specifically phenolic compounds on gut microbiota modulation.

4.1.4 Celery

Furanocoumarin, belonging to the family class coumarins, are phenolic compounds identified in celery according to the Phenol-Explorer database. However, another source identified the compounds apigenin and luteolin, from the flavone family class, in celery (34). An *in vivo* study also identified apigenin and luteolin as the most abundant flavonoids in celery when testing the use of celery juice and fermented celery juice to modulate gut microbiota in high-fat diet-induced obese mice (16). In another study testing bioactive compounds in celery, a new snack made of celery root was being developed consisting of celery root enriched with vegetable juices, including celery stalk juice (32). The initial phenolic compounds identified in celery root were: kaempferol, tannins, phenolic acids such as caffeic acid, ferulic acid, p-coumaric acid as well as flavonols, luteolin, and apigenin and saponin (32). Even though the part of the celery commonly used in smoothies is the stalks, not the celery root when celery stalk juice was added to the celery root snack, the phenolic content profile was enriched with two additional phenolic compounds which were apigenin derivatives and kaempferol derivatives different to the one already presents in the celery root (32). Considering that phenolic content can vary depending on the part of the vegetable used, it would be interesting to conduct further research on the phenolic content in the different parts of vegetables to determine which part (leaves, fruit, peel, flowers) would be the most beneficial to add to smoothies also keeping in mind the organoleptic quality.

4.1.5 Beetroot

Beetroot polyphenol composition includes phenolic acids, such as ferulic, vanillic, caffeic, and protocatechuic acids at a total concentration of 1513 mg/kg, and flavonoids, including catechin epicatechin, and rutin at concentrations of 386 mg/kg of beetroot pomace (35). The phenolic acid and flavonoid content in beetroot pomace was further confirmed in a study conducted to determine the antioxidant effects of beetroot pomace extract *in vivo* and *in vitro*. The phenolic compound in beetroot was identified and quantified by using high-performance liquid chromatography (HPLC) analysis. The results showed that beetroot polyphenols consisted of phenolic acids such as ferulic, hydroxybenzoic, caffeic, vanillic, and protocatechuic acids (36). Vulic J. et al (2013), state that phenolic compound concentrations in beetroot vary depending on the part of the vegetable being higher in the peel (50%) and lower in the flesh (13%) (36). This is interesting information to take into consideration as it would be ideal to include beetroot with the peel in smoothies as most phenolic compounds are contained in the peel. Further future studies could be done more specifically on phenolic compounds and their location within these vegetables.

4.1.6 Pumpkin

The phenolic compounds in pumpkin consist of the compound luteolin, belonging to the family class flavones, and a variety of lignans according to the Phenol-Explorer database. Studies on bioactive compounds, more specifically phenolic compounds in pumpkin are scarce. Most studies have been conducted on the carotenoid content in pumpkins (37). Researching bioactive compound composition in pumpkin is very complex as according to Kulczynski B et al (2019), this vegetable is the most diverse in terms of shape, color, and size (37). A study analyzing the different content of phenolic compounds in pumpkin confirmed that there exists a big diversity of phenolic acids and flavonols between pumpkin cultivars, but that all cultivars contain compounds belonging to the flavonols and phenolic acid family classes (37). Due to the lack of information on phenolic compounds in pumpkin and the heterogenicity among species and types of pumpkin, identifying concrete information on phenolic compounds is not clear and more research needs to be done on polyphenols in pumpkin. The results in Phenol-Explorer on phenolic compounds did not specify the specific species or cultivar so there will be no comparison of the results with other studies.

4.1.7 Carrot

The phenolic compound profile of carrots is characterized by the presence of phenolic acids having a concentration of 0.05 mg of hydroxybenzoic acid per 100 g of carrots and 20 mg of hydroxycinnamic acid derivatives per 100g of carrot, registered in the database Phenol-Explorer. A study evaluating the chemical composition, bioactive compound content, and antioxidant activity of fruits and vegetables found that total phenolic compounds in carrots resulted to be approximately 179.3 mg GAE (Gallic Acid Equivalents) per 100g of carrot while total flavonoid content resulted in 121.9 mg QE (Quercetin Equivalent) per 100g (13). In this same study, total phenolic compound and total flavonoid compound concentration were evaluated in beetroot and spinach too, resulting in beetroot having the highest total phenolic compound (909.5 mg GAE/100g) and total flavonoid content (221.6 mg QE/100g) of the three, followed by spinach which resulted in a total phenolic compound of 233.5 mg GAE/100g) and total flavonoid content, but had the lowest total flavonoid content of the three vegetables at a concentration of 108.7 mg QE/100g) (13).

4.2 Effects of Polyphenols on the gut microbiota

Bioactive compounds are non-nutrient components in plant-based foods that are known to exert anti-inflammatory and antioxidant effects which are beneficial for health (4). The vegetables in vegetable smoothie recipes are abundant in a variety of phenolic compounds such as flavonoid derivatives, flavonols, flavones, phenolic acids, tannins, lignans, and coumarins (**Table 1**). However, phenolic compounds from other food sources such as coffee, tea, berries, wine, grapes, etc., and their effect on modulating gut microbiota have been more frequently studied. These studies are of interest as relevant information has been found on the ability of bioactive compounds to modulate gut microbiota providing more information on the beneficial effects of polyphenols, some of which are also present in the selected studied vegetables.

4.2.1 Prebiotic effect

Phenolic compounds can exert prebiotic-like effects as they can selectively stimulate the growth of certain bacteria (3). When foods rich in flavonoids are consumed, these bioactive compounds reach the colon where they are metabolized and stimulate the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and inhibit the growth of pathogens (8). The results from different studies conducted on several sources of phenolic compounds such as flavonoid derivatives in cranberry extract and green tea, flavanols in cocoa, phenolic acids in red wine, and ellagitannins in pomegranate juice and pomegranate extract all showed prebiotic effects (19,21–25,27–29).

Results from different studies on the vegetables of interest also showed this prebiotic effect. For example, flavonoids in kale showed to exert this prebiotic effect in an *in vivo* study on HFD-induced obese mice, increasing *Bacteroidetes* and thus decreasing the *Firmicute/Bacteroidetes* ratio (14). A higher *Firmicute/Bacteroidetes* ratio is linked to a diet poor in fiber and high in saturated fat which is linked to microbiota diversity reduction and increased inflammation. Thus, lowering this bacteria ratio represents a healthier diet and is associated with lower levels of inflammation (14). Celery juice, contributing luteolin and apigenin, was studied *in vivo* on mice following an HFD (16). Resulted showed a prebiotic effect increasing *Lactobacillus* amongst other beneficial bacteria and decreasing the *Firmicute/Bacteroidetes* ratio (16). A study comparing the consumption of carrot dietary fiber containing carrot phenolic compounds vs de-phenolized carrot dietary fiber in mice, showed to exert a prebiotic effect on the gut microbiota, and by using both these substrates, the beneficial attributes can be more securely attributed to the polyphenols as it was the only difference between both substrates (18). Like the prebiotic effect from celery juice consumption, consuming carrot dietary fiber increased *Lactobacillus* as well as decreased the *Firmicute/Bacteroidetes* ratio, amongst other changes in microbiota composition.

This shows how the prebiotic effect is not attributed to only one family class of phenolic compounds but to a wide variety of compounds. It is also important to state how the prebiotic effect does not make the same type of bacteria grow, different bacteria growth can be stimulated as well as inhibited, and just because one food is specifically recognized for being abundant in one type of phenolic compound does not discard the fact that it could also contain other bioactive compounds that may contribute to the prebiotic effect.

4.2.2 Improved intestinal barrier function

The intestinal barrier is made up of a layer of epithelial cells, acting as a selectively permeable barrier, and a layer of mucus, enhancing gut immunity as it can secrete anti-microbial peptides. These layers act together to maintain gut homeostasis, allow for adequate nutrient absorption, and protect from external substances and pathogens (38). Intestinal homeostasis is influenced by gut microbiota diversity, composition, and essentially diet which influences gut microbiota (38).

Chronic inflammation is one of the main contributors to poor intestinal barrier function, thus the anti-inflammatory effects of polyphenols are important contributors as well to enhancing the intestinal barrier function (3)**.** Inflammation is also linked with a higher risk of developing a wide variety of diseases and evidence has shown how a healthy microbiota can influence diseases linked to inflammation as it can exert anti-inflammatory effects (3).

Polyphenols that have the capacity of exerting anti-inflammatory effects can do so by interfering in different biological inflammation signaling pathways, which will not be mentioned in detail in this review, but that have been proven in studies to decrease inflammatory cytokines in plasma serum levels (3).

Studies conducted in animals have shown how flavonoids can inhibit gastrointestinal inflammation (8). For example, cranberry extract has stood out for its rich content of flavonoids and studies have shown how its consumption in mice can inhibit pro-inflammatory signaling (8). This is also supported by the results of an *in vivo* study in humans with inflammatory bowel disease (IBD) who consumed mango pulp rich in phenolic acids, lowering the state of inflammation. Additional beneficial health implications were witnessed as the state of IBD improved (25). Aronia melanocarpa also exerted an anti-inflammatory effect, significantly lowering intestinal inflammation in rats with induced colitis and further proved by decreased inflammatory serum markers (19). In a study where mice submitted to an HFD and became obese, the consequences were gut microbiota dysbiosis, lowered microbiota diversity, and increased *Firmicute/Bacteroidetes* ratio. Quercetin and kaempferol from Kale were administered to the obese mice resulting in reversed gut microbiota dysbiosis by reducing the firmicute/Bacteroidetes ratio and increased total microbiota diversity (14). Obesity also creates a state of inflammation, and dysbiosis so polyphenols in kale enhanced good metabolic function and essentially lowering inflammation levels (14).

One of the mechanisms by which the microbiota may protect the host from inflammation of the intestinal mucus is due to the production of SCFA by certain bacteria that make up the gut microbiota while on the other hand pathogenic bacteria such as E. coli, worsen inflammation (25). SCFAs are produced by beneficial bacteria such as *Prevotella*, *Lactobacillus*, *Bacteroidetes,* and *Bifidobacterium* in the gut. These bacteria are necessary for optimal intestinal barrier function as SCFAs are the main energy source for the intestinal epithelial cells and allow them to maintain the integrity of the intestinal barrier (6,7). Thus, if the growth of SCFAs producing bacteria is stimulated by consumption of foods high in polyphenols then the intestinal barrier function will be enhanced. Quercetin supplementation with a 98% purity was administered to diet-induced obese mice and increased the production of SCFA, prebiotic effect, and lessened gut dysbiosis, enhancing intestinal barrier function (17). This positive effect might apply to foods high in quercetin, but future studies need to be conducted as in foods, quercetin bioavailability and metabolism would be different. Beetroot, containing quercetin along with a variety of phenolic acids increased SCFA production in an *in vivo* study on healthy humans (17). An increase in SCFA production was also witnessed in studies conducted on carrot dietary fiber polyphenols *in vivo* in mice and *in vitro* on a human fecal sample (18) and in another *in vivo* study conducted on mice eating spinach (15).

4.3 Limitations

Although polyphenols appear to be the perfect solution to achieve optimal gut health and overall health and reduce the risk of developing chronic diseases, it is not as simple as it sounds. Polyphenol metabolism and utilization by the gut microbiota depends on many factors such as bioavailability, chemical structure, etc. (3). For example, according to Dominguez-Avila et al (2021), a big source of phenolic compounds is coffee which is popularly consumed worldwide but the phenolic compound concentration in coffee can vary significantly depending on factors such as the processing of the beans, the environment, and genetics (3). Roasting the coffee beans, which is a very frequent process in coffee preparation to enhance its sensorial qualities appears to deplete approximately 90% of chlorogenic acids (phenolic acids in coffee), which one would assume would be benefiting largely from (3). Research on the bioavailability of bioactive compounds in vegetables used in smoothies needs to be done, to gather more data on the effects of these compounds on the gut microbiota and be able to affirm the magnitude of the effect on modulation of the gut microbiota. If the phenolic compounds are not bioavailable, then these compounds cannot be utilized by the body, so finding more information on this is very important. Another limitation of this review is that studies on the phenolic compounds in the vegetables of interest in this review are scarce so little information is available to make concrete conclusions on the influence that these vegetables have on the gut microbiota. In pumpkin and cucumber specifically, no studies were found on how phenolic compounds in these vegetables modulate gut microbiota, so no conclusions can be made regarding these. In addition, there are little to no clinical trials performed on humans to see how polyphenols modulate the human gut microbiota, most studies were in vivo conducted on animals such as rats as well as an in vitro, making it more complicated to extrapolate the results to humans. These are some limitations that should be addressed to further understand the interplay between food, bioactive compounds, and gut microbiota. It is also important to state that the diet is very complex, and it is almost nearly impossible to attribute the beneficial effects observed in the gut microbiota to only polyphenols.

5 Conclusion

All selected vegetables as the most common to prepare smoothies, showed to have a variety of bioactive compounds, including carotenoids, pigments, and phenolic compounds in their composition. The most abundant polyphenols were kaempferol derivatives and quercetin in kale, flavonols in spinach, quercetin and kaempferol in cucumber, furanocoumarins in celery, luteolin in beetroot, and pumpkin, and phenolic acids in carrot. Out of all the vegetables studied kale, spinach, cucumber, celery, beetroot, and carrots showed to modulate the gut microbiota, exerting a prebiotic effect, and increasing SCFA production, while no evidence has been found for pumpkin and cucumber of having any effects on the gut microbiota. Polyphenols from other food sources (*Aronia melanocarpa* berry, mango pulp, and cranberry extract) have shown anti-inflammatory effects on the gut microbiota enhancing intestinal barrier function, but no evidence was observed from studies conducted on the vegetables used in smoothies. Last, but not least, although more studies need to be conducted on vegetable smoothies and their potential for gut microbiota modulation, the consumption of vegetable smoothies might help reach daily vegetable servings and contribute important nutrients and bioactive compounds, supporting a healthy diet.

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