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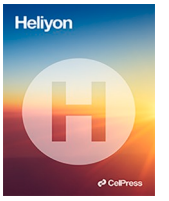

Review article

Uncovering the promising role of grape pomace as a modulator of the gut microbiome: An in-depth review☆

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ABSTRACT

Grape pomace is the primary wine coproduct consisting primarily of grape seeds and skins. Grape pomace holds immense potential as a functional ingredient to improve human health while its valorization can be beneficial for industrial sustainability. Pomace contains bioactive compounds, including phenols and oligosaccharides, most of which reach the colon intact, enabling interaction with the gut microbiome. Microbial analysis found that grape pomace selectively promotes the growth of many commensal bacteria strains, while other types of bacteria, including various pathogens, are highly sensitive to the pomace and its components and are inactivated. *In vitro* studies showed that grape pomace and its extracts inhibit the growth of pathogenic bacteria in *Enterobacteriaceae* family while increasing the growth and survival of some beneficial bacteria, including *Bifidobacterium* spp. and *Lactobacillus* spp. Grape pomace supplementation in mice and rats improves their gut microbiome complexity and decreases diet-induced obesity as well as related illnesses, including insulin resistance, indicating grape pomace could improve human health. A human clinical trial found that pomace, regardless of its phenolic content, had cardioprotective effects, suggesting that dietary fiber induced those health benefits. To shed light on the active components, this review explores the potential prebiotic capacity of select bioactive compounds in grape pomace.

1. Introduction

Grapes are a leading agricultural commodity largely grown for wine production. According to a recent FAO document [1], the estimated total grape production worldwide in 2021 was 73,524,196 tons, with the five largest producers being China (11.2 million tons), Italy (8.15 million tons), Spain (6.09 million tons), United States of America (5.49 million tons) and France (5.07 million tons), respectively. The four largest wine-producing countries are Italy, France, Spain, and the United States, with 260 million hectoliters of wine produced worldwide in 2021, and with world wine production expected to remain stable in 2022 despite the drought and heat waves recorded in wine regions around the world [2,3]. In California alone, 3.88 million tons of grapes were crushed in 2021 [4]. Wine grapes undergo a specific set of steps for wine-making. Broadly, grapes are harvested, destemmed, crushed, and macerated to release the juice. For white wines, the grapes are immediately pressed to separate the solids from the juice, which is then fermented. For red

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wines, the skins are fermented with the juice and pressed at a later stage. Both white and red wines are then further fermented, clarified, matured, stabilized, and filtered before bottling. Fermentation of red grape solids (stems and seeds) with the grape juice has the potential to alter their composition.

Wine production encompasses a large portion of agriculture as well as local and global economies, however, it generates massive quantities of agricultural waste. Grape pomace (also called grape marc) is considered an agro-industrial waste and the primary solid by-product of the wine industry and consists of skins, seeds, and stalks left over after wine-making, representing about 10–30 % (w/w) of the processed grape weight, depending on the grape variety, viticulture practices, and wine-making process [5–7]. Grape pomace is still primarily disposed of through composting or utilized as an economic livestock feed, therefore, it holds great potential for valorization in higher end applications. Both white and red grape pomaces contain large amounts of indigestible fiber, including polysaccharides and oligosaccharides, as well as phenolic compounds, a class of compounds with antioxidant activity and more. The potential health impact of grape pomace has become an increasingly investigated topic, primarily regarding its phenolic content and overall effect on the gut microbiome. The gut microbiome consists of a complex collection of trillions of bacteria, both commensal and pathogenic, which colonize the intestines [8]. These bacteria break down food components that are indigestible to humans into smaller metabolites, which can often be absorbed by the intestines or otherwise utilized. Commensal bacteria also help maintain homeostasis by preventing intestinal infection from pathogens, thus reducing the burden of severe disease and concomitant inflammation and producing beneficial short-chain fatty acids [9].

The market for grape pomace and seeds as health supplements has expanded with growing research showing its health benefits. While a few new products incorporate whole grape pomace into foods like chocolate bars and breads, the majority is sold as

Table 1
Phenolic compounds in grape pomace.

Phenolic compound	Malbec (red) grape pomace extract (μg/g) [22]	Chardonnay pomace (μg/g dry weight) [21]	Vitaflavan® grape seed extract (mg/g) [23]	Cencibel grape pomace (mg/mL) [24]	Voignier pomace extract (mg/g) [25]	Vidal Blanc pomace extract (mg/g) [25]	Cabernet Franc pomace extract (mg/g) [25]	Chambourcin pomace extract (mg/g) [25]
Gallic acid	252.8 ± 18.5	116 ± 2	9.11 ± 0.10	99.6				
Vanillic acid		15 ± 2						
Syringic acid	1731.7 ± 156.3							
Caffeic acid	16.0 ± 2.6			100.5				
(+)-Catechin	3387.5 ± 374.7	670 ± 10	74.54 ± 0.09	99.6	910 ± 10.5	631 ± 13.4	560 ± 4537	214 ± 4.80
(−)-Epicatechin	1763.4 ± 221.8	890 ± 30	67.68 ± 0.75	99.9	625 ± 9.20	451 ± 22.2	215 ± 4.67	109 ± 4.17
(−)-Gallocatechin		1490 ± 40						
Epicatechingallate					427 ± 11.7		122 ± 2.99	56.9 ± 5.36
(−)-Epigallocatechin		250 ± 4						
(−)-Epigallocatechin gallate		24.2 ± 0.2			96.1 ± 3.47	62.8 ± 0.78	171 ± 7.26	
(−)-Epicatechin gallate		28 ± 1						
(−)-Epicatechin-3-*O*-gallate			26.21 ± 0.41					
Gallocatechin gallate					99.1 ± 1.29		232 ± 3.19	146 ± 3.37
Trans-resveratrol		26.3 ± 0.5						
Quercetin-3-glucoside	112.2 ± 12.1							
Quercetin-3-rhamnoside					27.1 ± 2.59	33.5 ± 1.57		
Quercetin	557.3 ± 83.9			100.9	17.3 ± 0.38	20.7 ± 0.01	56.5 ± 1.95	31.2 ± 2.26
Rutin					255 ± 16.7	435 ± 14.0	343 ± 11.0	99.5 ± 0.39
Tyrosol	34.0 ± 2.7							
Trans-resveratrol		26.3 ± 0.5						
Procanidin B3			20.39 ± 0.33					
Procyanidin B1			60.99 ± 1.42					
Procyanidin T2			6.81 ± 0.06					
Procyanidin B4			15.04 ± 0.13					
Procyanidin B2			45.13 ± 0.95					
Procyanidin C1			7.07 ± 0.08					
B1-3-*O*-gallate			0.32 ± 0.04					
B2-3-*O*-gallate			1.80 ± 0.06					
B2-3′-*O*-gallate			1.61 ± 0.00					

supplements featuring grape pomace or seed extracts in the form of pills or sachets. These supplements are marketed for their antioxidant properties and associated health benefits [10–12]. Much of the recent research on grape pomace focuses on optimizing the extraction of health-promoting compounds from grape pomace to further improve the impacts of grape pomace applications like these supplements. As further progress is made through extraction techniques like high hydrostatic pressure extraction [13], ultrasound-assisted extraction [14], microwave-assisted extraction [15], and enzyme-assisted extraction [16], supplement companies could obtain a higher phenolic recovery and potentially develop supplements for particular functions or target populations.

A prebiotic is defined as "a substrate that is selectively utilized by the host's microorganisms conferring a health benefit," [17] whereas probiotics are the beneficial bacteria that provide desirable health benefits and consume the prebiotics *in situ.* Prebiotic and probiotic supplements have become increasingly prevalent as knowledge of and emphasis on the importance of maintaining the gut microbiome on overall health has increased. This review surveys the current literature on grape pomace phenolics and oligosaccharides and the valorization potential of grape pomace as a source of compounds able to modulate gut health and decrease diseases through *in vitro*, GI tract simulation, small animal, livestock and human studies. In the near future, it is expected that novel products leveraging grape pomace natural functional capabilities as a microbiome modulator or prebiotic-probiotic combination supplements will enter the market.

2. Prebiotics in grape pomace

Recent research has investigated two classes of prebiotic compounds in grape pomace, phenolics and oligosaccharides. Both phenolics and oligosaccharides have been reported to act as prebiotics by selectively inhibiting pathogen growth and binding to host-cells, while also promoting the growth of beneficial gut bacteria [18,19].

2.1. Phenolic compounds: composition and distribution

Phenolic compounds are largely known for their antioxidant capabilities but have also been reported to exert antimicrobial, anticancer, anti-inflammatory, antidepressant and antithrombotic effects and reduce the risk of diabetes, cardiovascular, carcinogenic and neurodegenerative diseases [6]. They encompass a large catalogue of compounds naturally found in plants. Grape pomace is considered a rich source of phenolic compounds since only 30–40 % of grape's phenolic compounds are extracted during wine-making, with the remaining ending up in the pomace [7]. Phenolic compounds present in grape pomace can be classified as simple phenols (*p*-coumaric acid, ferulic acid, caffeic acid, sinapic acid; and gallic acid, protocatechuic acid, syringic acid, vanillic acid and sinapic acid as hydroxycinnamic acids and hydroxybenzoic acids, respectively) and polyphenols. The latter can be further classified in flavonoids (flavonols such as kaempferol, quercetin, myricetin, flavanols as catechin, epicatechin, epigallocatechin, gallocatechin, anthocyanidins such as petunidin, peonidin, maldivin, delphinidin, cyanidin), stilbenes (resveratrol), tannins (proanthocyanidins, gallotannins, ellagitannins) [6,20]. The concentrations and types of each phenolic compound in grape pomace can vary in pomace from different grape cultivars or methods of processing. Grape seeds are known to contain higher total phenolic than grape skins. The primary phenolic compound in chardonnay grape seeds, (−)-epicatechin, is not present in the skins, while the most concentrated phenolic in chardonnay grape skins, (−)-gallocatechin, is not found in the seeds [21]. The grape variety also has an important impact. For example, (+)-catechin and (−)-epicatechin were found in all the varieties detailed in Table 1, while caffeic acid was only identified in Malbec and Cencibel pomace. Similarly, Sinrod et al. [21] found (−)-gallocatechin to be the most abundant phenolic in chardonnay

Table 2
Oligosaccharides in grape pomace.

Material	Oligosaccharide composition	Source
Nebbiolo red grape seeds	Hex_3, Hex_4, Hex_5, Hex_6, Hex_7, Ara_8, Ara_9, Ara_10, Ara_11, GalA-Rha-Ara_7, GalA- Rha-Ara_8, GalA-Rha-Ara_9, GalA-Rha-Ara_10, GalA-Rha-Ara_11, GalA-Rha-Ara_12, GalA-Rha-Ara_13, GalA-Rha- Ara_14, GalA-Rha-GalA-Ara_5, GalA-Rha-GalA-Ara_6, GalA-Rha-GalA-Ara_7, GalA-Rha-GalA-Ara_8, GalA-Rha-GalA-Ara_9, GalA-Rha-GalA-Ara_10, GalA-Rha-GalA-Ara_11, GalA-Rha-GalA-Ara_12, GalA-Rha-GalA-Ara_13, GalA-Rha-GalA-Ara_14, GalA-Rha-GalA-Ara_15	[30]
Chardonnay pomace	Hex_3, Hex_4, Hex_5, Hex_2 Pent_1, Hex_3 Pent_2, Hex_3 Pent_1 HexA_1, Hex_3 Pent_2 HexA_1, Hex_3 Pent_3 HexA_1, Hex_6, Hex_2 HexA_1, Hex_3 HexA_1, Pent_4 HexA_1, Pent_5 HexA_1, Hex_2 Pent_2, Hex_2 Pent_3, Pent_3, dHex_1 Pent_1, Pent_2 HexA_1, dHex_1 Pent_1 HexA_1, Hex_1 Pent_1 HexA_1, Hex_2 dHex_1, Hex_1 dHex_1 HexA_1, dHex_2 methyl-HexA_1, HexA_3, Pent_4, dHex_1 Pent_3, dHex_1 Pent_2 HexA_1, Pent_2 Methyl-HexA_1 Xylitol_1, Hex_2 dHex_2, Hex_2 Pent_1 HexA_1, dHex_2 HexA_2, Hex_2 dHex_1 HexA_1, Pent_5, Pent_3 Methyl-HexA_1 Xylitol_1, dHex_1 Pent_3 HexA_1, Pent_3 HexA_1, dHex_3 HexA_2, Pent_4 Methyl-HexA_1 Xylitol_1, Hex_4 Pent_2, dHex_3 HexA_3, Hex_1 Pent_2 HexA_1	[21, 31]
Chardonnay skins	Hex_3, Hex_4, Hex_6, Hex_7, Hex_2 Pent_1, Hex_2 Pent_2, Hex_2 Pent_3, Hex_3 Pent_1, Hex_4 Pent_1, Hex_4 Pent_2, Hex_5 Pent_1, Hex_2 HexA_1, Pent_4 HexA_1, Pent_5 HexA_1, Hex_2 HexNAc_1, Hex_3 HexNAc_1, Hex_4 HexNAc_1, Hex_5 HexNAc_1, Hex_2 Pent_3 HexA_1	[21]
Chardonnay seeds	Hex_3, Hex_4, Hex_5, Hex_6, Hex_7, Hex_8, Hex_2 HexA_1, Pent_5 HexA_1, Hex_2 Pent_1, Hex_2 Pent_2, Hex_2 Pent_3, Hex_3 HexNAc_1, Hex_4 HexNAc_1, Hex_1 HexNAc_1 HexA_1, Hex_3 Pent_2 HexA_1	[21]
Chardonnay seed extract	Hex_3, Hex_4, Hex_5, Hex_6, Hex_7, Hex_8, Hex_9, Hex_2 Pent_1, Hex_2 Pent_2, Hex_2 Pent_3, Hex_3 HexNAc_1, Hex_4 HexNAc_1, Hex_3 HexA_1, Hex_3 HexA_2, Hex_4 HexA_3, HexNAc_3, Hex_1 HexNAc_2 HexA_1, Hex_2 HexNAc_1 Pent_1, Hex_3 HexNAc_1 Pent_1, HexNAc_4 Pent_1 HexA_2	[21]

Monosaccharides are listed with their abbreviated names, followed by the number of units in which they appear in each oligosaccharide. Hex: Hexose (glucose, galactose, mannose); Ara: arabinose; GalA: galacturonic acid; Rha: rhamnose; Pent: pentose (arabinose, xylose); HexNAc: N-Acetyl-hexosamine.

pomace, but that compound was not identified in any of the other varietals (Table 1).

2.2. Oligosaccharides: composition and distribution

Oligosaccharides are a class of non-digestible carbohydrates containing 3 and 20 monosaccharide building blocks joined by a diversity of glycosidic residues. Multiple types of oligosaccharides have been identified in grapes, wine, and wine coproducts in recent studies. Blanch et al. [26] found 5 fructo-oligosaccharides (1-kestose, neokestose, nystose, nystose b, and kestopentaose) in red table grapes, while Dos Santos Lima et al. [27] identified 1-ketose, nystose, and raffinose oligosaccharides in grape juice and wine. Oligosaccharides are present in both red and white wines, as shown in the study conducted by Bordiga et al. [28], who reported 45 distinct oligosaccharides in wine with degrees of polymerization of 3–14 monosaccharide building blocks made of glucose, arabinose, xylose, mannose, rhamnose, fucose, galacturonic acid, and glucuronic acid. Oligosaccharides containing arabinose, mannose, galactose, glucose, and rhamnose have been found in seeds isolated from red wine pomace (Table 2) [29]. Furthermore, our recent study of chardonnay pomace, its seed and skin components, and a seed extract collectively found 36 distinct oligosaccharides between the samples that were composed of 11 different building blocks, including hexoses, pentoses, N-acetylhexosamine, and hexuronic acid monosaccharides [21]. While significant overlap in terms of oligosaccharides presence/abundance existed among the chardonnay pomace fractions, each fraction had a set of unique oligosaccharides not found in the other fractions. The skins had the largest number of naturally occurring oligosaccharides and the most unique oligosaccharides compared to the other fractions. Interestingly, applying subcritical water extraction to chardonnay seeds increased the number of oligosaccharides obtainable from the seeds [21].

3. Investigating the effect of grape pomace on microbiota

The importance of gut health is becoming increasingly studied and known. One of the key determinants of gut health is maintaining a balanced gut microbiome that includes sufficient amounts of commensal bacteria such as *Bifidobacterium longum, Lacticaseibacillus rhamnosus, Lactiplantibacillus plantarum* and *Lactobacillus acidophilus* [32]. One popular method for improving the gut microbiome is through probiotic supplements where viable commensal bacteria are consumed to repopulate the intestines. However, to be effective, these bacteria must remain viable after passing through the harsh conditions of the upper GI tract and then must be able to remain in the gut and consume the carbon sources available *in situ*. Eight strains of commonly used probiotic bacteria demonstrated rapid decreases in viability when exposed to *in vitro* acidic conditions that replicated those of the stomach [32]. Ingested probiotic bacteria are also exposed to bile salts, including oxgall and taurocholic acid in the upper GI tract. Similarly to stomach acid, oxgall and taurocholic bile acids significantly reduce probiotic viability [32]. The loss of probiotic viability during digestion decreases or eliminates the ability of probiotics to colonize the gut with beneficial bacteria. To address this challenge, probiotics can be co-delivered with compounds that not only help protect them from the upper GI tract harsh conditions but also selectively feed these bacteria to aid colonization while hindering the growth of less desirable bacteria already present in the gut. Initial *in vitro* studies indicate that grape pomace possesses this functionality. When co-delivered with grape pomace, the *in vitro* survival of certain lactic acid bacteria and bifidobacteria (*L. plantarum* 12A, *L. plantarum* PU1, *Lacticaseibacillus paracasei* 14A, *B. breve* 15A) was shown to increase during exposure to GI tract conditions, indicating that grape pomace is an effective food matrix to deliver probiotics to the colon [33].

In a further evaluation of grape pomace phenolics and oligosaccharides as prebiotics, the "diet" of a GI simulator was supplemented with grape pomace, resulting in an improved composition and functionality of its "artificial" gut microbiome, generated with fecal inoculum from two healthy human donors, in every section of the colon. The abundance of *Lactobacillus*, *Bacteroides*, *Bifidobacterium*, *Enterococcaceae*, *Clostridia XIVa*, *Enterobacteriaceae*, and *Faecalobacterium prausnitzii* derived from healthy human feces increased with chronic grape pomace feeding. Of these, *Lactobacillus*, *Bacteroides*, *Clostridia XIVa*, *Enterobacteriaceae*, and *Faecalibacterium pausnitzii* growth increased the most [34]. Unfortunately, the specific components of the grape pomace that led to this increased growth were not characterized and since the pomace contained simple sugars, oligosaccharides, and phenolics, the active ingredient could not be identified in that work.

Gastrointestinal tract simulation studies illustrate that the phenolics present in grape pomace and many oligosaccharides possess enough stability to reach the large intestine intact in sufficient concentrations to affect the gut microbiome. While interacting with phenolics, commensal bacteria convert large phenolics into metabolites that are more readily absorbed by the intestines, increasing the biological functionality of grape pomace while improving the composition of the gut microbiome. These studies further support the valorization of grape pomace as a functional ingredient.

Determining the bioaccessibility of phenolics and oligosaccharides in grape pomace is crucial to evaluating their potential health benefits. Bioaccessibility is defined as the release of a compound from the food matrix during gastrointestinal digestion [35], which then becomes available for absorption into the body to produce its health effects. Grape pomace phenolics and oligosaccharides must be released from the matrix and survive or transform into prebiotic compounds in the harsh environment of the digestive tract to exert beneficial effects in the intestines. Simulated gastrointestinal digestion enables scientists to closely monitor the effects of each stage of digestion as well as the stabilities of individual compounds.

3.1. The effect of grape pomace phenolic compounds on microbiota

Although some methods such as micro- and nanoencapsulation have been developed to increase the bioavailability of phenolic compounds, absorption in the small intestine is very low (5–10 %) and 90–95 % can reach the colon because of insufficient gastric residence time and low permeability [35,36]. The main metabolites are formed from polyphenols by the cleavage of ester and

glycoside bonds, ring-fission, and chemical modifications, producing different benzoic acids, phenylacetic acids, phenylpropionic acids, valerolactones and cinnamic acids, and these new substances may undergo phase I/II transformations performed by gut epithelial cells and hepatocytes [37,38]. Polyphenols are biotransformed by the gut microbiota, firstly by deglycosylation, followed by the breakdown of flavonoids into relatively simple aromatic carboxylic acids (phenolic acids) [39]. For example, anthocyanidins are metabolized to form 2,4,6-trihydroxyphenylacetic acid and protocatechuic acid, proanthocyanidins are transferred to 3,4-dihydroxyphenylacetic acid, 3-(3-hydroxyphenyl) propionic acid, 3-hydroxyphenylacetic acid and 5-(3′-hydroxyphenyl)-γ-valerolactone; and quercetin 3-*O*-glucoside is converted to phloroglucinol, 2,4,6-trihydroxybenzoic acid, and protocatechuic acid by the intestinal bacteria [35].

Bacterial metabolism transforms grape pomace phenolics into more bioavailable metabolites like 3,5-dihydroxybenzoic acid and phenylacetic acid, which can be absorbed into the body [34]. There, (−)-epigallocatechin gallate, a prominent phenolic compound in grape pomace, is broken down by either *Raoultella ornithinolytica* or *Raoultella planticola* as demonstrated with an *in vitro* dynamic gastrointestinal digestion model [34]. After surviving the simulated upper GI tract, the phenolic content of the grape pomace digestate increases during the intestinal phases. The gut microbes from the fecal inoculum induce this increase through two mechanisms. First, enzymes produced by gut microbes release phenolics bound to the cell walls and dietary fiber in the grape matrix and this makes the phenols bioavailable and detectable [34,40]. Second, gut microbes metabolize phenolics into metabolites that are more bioactive than the original larger polyphenols. This was determined as the phenolics originally present in the grape pomace, including (+)-catechin and (−)-epicatechin, decrease significantly with digestion, while smaller phenolics that were not in the original pomace are detected in the digestate [40,41]. Furthermore, 21 grape pomace phenolic metabolites were identified, including benzoic acids, phenols, phenylpropionic acids, phenlyactic acids, cinnamic acids, valeric acids, and valerolactones. The abundance of these metabolites decreased when grape pomace supplementation stopped, indicating that they were generated during the microbial metabolism of the pomace [34]. The smaller grape pomace phenolic metabolites were absorbed by the large intestine and provided at least some of the health benefits associated with grape pomace consumption [34]. Scientists have determined how some bacteria in the human gut microbiome

Table 3
The responses of pathogenic bacteria to grape pomace and grape pomace extracts measured in various *in vitro* studies (DF: Dietary Fiber, GPE: Grape Pomace Extract, GSE: Grape Seed Extract, Monos: Monosaccharide, N/A: Not Available, POS: Polysaccharide, TPC: Total Phenolic Content, XOS: Xylooligosaccharides).

Bacteria	Growth Effect	Pomace Intervention	Study Type	Measured Carbohydrates	Measured Phenolics	Source
Clostridia XIVa	Increased	GPE	GI tract simulator	Extract DF and Monos	Extract TPC and individual phenolics	[34]
Escherichia coli ATCC 25922	Decreased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC and individual phenolics before and after incubation	[45]
E. coli ATCC 25922	No effect	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
E. coli ATCC 35218	Increased	GPE	*In vitro* growth assay	N/A	Extract TPC	[48]
E. coli BW13711	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
E. coli CECT 5947	Increased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
E. coli WTT1	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
Enterococcaceae	Increased	GPE	GI tract simulator	Extract DF and Monos	Extract TPC and individual phenolics	[34]
Enterobacteriaceae	Increased	GPE	GI tract simulator	Extract DF and Monos	Extract TPC and individual phenolics	[34]
Faecalibacterium prausnitzii	Increased	GPE	GI tract simulator	Extract DF and Monos	Extract TPC and individual phenolics	[34]
Pseudomonas aeruginosa ATCC 10145	Decreased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC and individual phenolics before and after incubation	[45]
Streptococcus salivarius ZL50-7	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
S. salivarius ZL93-3	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
S. sobrinus ATCC 33478	No effect	GPE	*In vitro* growth assay	N/A	Extract TPC	[48]
S. thermophilus STY-31	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
Staphylococcus aureus ATCC 25923	Decreased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC and individual phenolics before and after incubation	[45]
S. aureus CCUG 60578	Decreased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC and individual phenolics before and after incubation	[45]

Table 4
The responses of specific gut bacteria to grape pomace and grape pomace extracts measured in various *in vitro* studies (CHO: Carbohydrate, DF: Dietary Fiber, GPE: Grape Pomace Extract, GSE: Grape Seed Extract, Monos: Monosaccharides, N/A: Not available, POS: Polysaccharides, RGP: Red Grape Pomace, TPC: Total Phenolic Content, XOS: Xylooligosaccharides).

Bacteria	Growth Effect	Pomace Intervention	Study Type	Measured Carbohydrates	Measured Phenolics	Source
Bifidobacterium animalis 13A	Decreased	RGP	*In vitro* growth assay	N/A	N/A	[33]
B. breve 15A	Increased	RGP	*In vitro* growth assay	Total CHO before and after incubation	TPC, individual phenolics before and after incubation	[33]
Bacteroides	Increased	GPE	GI tract simulator	Extract DF and Monos	Extract TPC and individual phenolics	[34]
Bifidobacterium	Increased	GPE	GI tract simulator	Extract DF and Monos	Extract TPC and individual phenolics	[34]
B. animalis Bo	Increased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC and individual phenolics before and after incubation	[45]
B.animalis spp. Lactic Bb12	Increased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC and individual phenolics before and after incubation	[45]
B. longum BG3	Increased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC and individual phenolics before and after incubation	[45]
Bifidobacterium spp. ATCC 29521	Increased	GPE	*In vitro* growth assay	N/A	Extract TPC	[48]
Lactobacillus acidophilus ATCC 43121	No effect	GPE	*In vitro* growth assay	N/A	Extract TPC	[48]
L. acidophilus CECT 903	Increased	RGP extract	*In vitro* growth assay	N/A	Extract TPC, individual phenolics	[24]
Lacticaseibacillus casei FC1-13	Decreased	RGP	*In vitro* growth assay	N/A	N/A	[33]
L. casei FPL7190	Increased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. casei LC-01	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
Limosilactobacillus fermentum LC-40	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. fermentum PNA1	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
Lacticaseibacillus paracasei 14A	Increased	RGP	*In vitro* growth assay	Total CHO before and after incubation	TPC, individual phenolics before and after incubation	[33]
Lactiplantibacillus plantarum 12A	Increased	RGP	*In vitro* growth assay	Total CHO before and after incubation	TPC, individual phenolics before and after incubation	[33]
L. plantarum CIC17	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. plantarum CLB7	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. plantarum IFPL711	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. plantarum IFPL715	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. plantarum IFPL722	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. plantarum IFPL724	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. plantarum IFPL935	Decreased	GSE	*In vitro* growth assay	N/A	Extract individual phenolics	[23]
L. plantarum PU1	Increased	RGP	*In vitro* growth assay	Total CHO before and after incubation	TPC, individual phenolics before and after incubation	[33]
Limosilactobacillus reuteri DSM20016	Decreased	RGP	*In vitro* growth assay	N/A	N/A	[33]
Lacticaseibacillus rhamnosus SP1	Decreased	RGP	*In vitro* growth assay	N/A	N/A	[33]
Furfurilactobacillus rossiae DSM15814	Decreased	RGP	*In vitro* growth assay	N/A	N/A	[33]
Lactobacillus	Increased	GPE	GI tract simulator	Extract dietary fiber and Monos	Extract TPC and individual phenolics	[34]
L. casei 01	Increased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC, individual phenolics before and after incubation	[45]

(*continued on next page*)

Table 4 (*continued*)

Bacteria	Growth Effect	Pomace Intervention	Study Type	Measured Carbohydrates	Measured Phenolics	Source
L. rhamnosus R11	Increased	Enzymatic GPE	*In vitro* growth assay	Total DF, Monos, XOS, and POS quantification before and after incubation	TPC, individual phenolics before and after incubation	[45]

interact with flavan-3-ols like (−)-epicatechin gallate and (−)-epigallocatechin gallate, which are present in grape pomace. Human fecal bacteria produce esterases, which cleave the flavan-3-ol's salic acid ester (gallic acid), which is decarboxylated into pyrogallol. The carbon ring of flavan-3-ol is then opened to form diphenylpropan-2-ol, which is converted to 5-(3′, 4′-dihydroxyphenyl)-γ-valerolacetone. The valerolacetone ring is broken to generate 5-(3′, 4′-dihydroxyphenyl) valeric acid and 4-hydroxy-5-(3′, 4′-dihydroxyphenyl) valeric acid. These compounds are dehydroxylated into mono-hydroxylated phenolic acids which are absorbed through the intestine and metabolized by the liver [42–44].

Multiple studies have determined that grape pomace phenolics partially degraded before reaching the large intestine [45,46]. This is in part due to the fact that many phenolics are pH-sensitive, making them vulnerable to gastric acid. Both flavanol and anthocyanin contents decreased after passing through the stomach section of the simulator. The gastric acid and pancreatic conditions of this segment likely induced the degradation. Wang et al. [47] researched the effects of *in vitro* gastrointestinal digestion on the phenolic content and antioxidant capacity of red grape pomace, and they found that phenolic compounds were stable under gastric conditions, however, there was an important decrease in the total phenolic content and antioxidant activity after pancreatic digestion, and anthocyanins and flavonols were more sensitive to the conditions simulating the small intestine.

Gil-Sánchez et al. [34], however, found that large polyphenols reached the large intestine intact. Additionally, the gastric acid conditions in the stomach degraded grape pomace oligosaccharides [45]. Encapsulating grape antioxidants and oligosaccharides is a potential method to increase the amount of the potentially bioactive compounds that reach the large intestine for microbial fermentation. Alginate encapsulation of grape pomace extract increases the concentration of intact phenolics in the large intestine where they were released with fermentation [40]. The food matrix delivering bioactive compounds could also be optimized to improve phenolic and oligosaccharide stability, thus decreasing their degradation during the early stages of digestion without encapsulation.

Grape pomace and its extracts have been shown to repress the growth of several strains of pathogenic bacteria; the results of these studies are summarized in Table 3. Oligomer phenolic compounds have been shown to possess a stronger antimicrobial effect than monomeric phenolics towards pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus epidermis*, and *Enterococcus faecalis* [23]. Several of the oligomeric phenolic compounds in grape pomace have been shown to specifically prevent the growth of pathogens. For example, catechins hinder *Escherichia coli*, *Bacillus cereus*, and *Serratia marcescens* growth; gallic acid is antimicrobial for *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa*; quercetin suppresses *E. coli*, *Proteus mirabilis*, and *Klebsiella pneumoniae* growth; and caffeic acid is antibacterial for *E. coli*, *P. aeruginosa*, *S. aureus*, and *P. mirabilis* [22–24]. Karamati Jabehdar et al. [48] tested the ability of a variety of bacterial strains to grow on grape pomace phenolic extract and found that while *E. coli* ATCC 35218 was able to grow at every tested concentration of grape pomace. The growth of *Streptococcus* spp. was inhibited when exposed to the extract (Table 3) [48], suggesting strain level specificity in terms of antimicrobial function as well.

Grape pomace supplementation can also enhance the growth and survival of some commensal bacteria strains. Studies have shown that certain commensal bacteria metabolize grape phenolic compounds and, therefore, are able to grow when exposed to grape pomace, as depicted in Table 4. *Lactobacillus* and *Bifidobacterium* are the most widely used genera in commercial probiotics, and a few studies indicated that in some specific cases, their growth can be stimulated by grape phenolics. For example, the growth of *B. breve* 26M2 and *B. bifidum* HDD541 increased when exposed to grape seed extract, whereas *B. lactis* BB12 appeared to be sensitive to the phenolic extracts [23]. Similarly, while *L. plantarum* IFPL935 was able to metabolize the phenolic compounds and reach maximal growth, certain other lactobacilli like *Limosilactobacillus fermentum*, *L. acidophilus,* and *Limosilactobacillus vaginalis* exhibited high sensitivity to the phenolic extracts. Additionally, lactic acid bacteria and bifidobacteria have shown increasing growth with increasing total concentrations of phenolics and procyanidin, a phenolic compound that is also found in grape seed extracts. It appears that the sensitivity of gut bacteria to grape pomace and grape seed phenolics varies greatly depending on the specific strain. As additional examples, *L. plantarum* IFPL 724 and *Lacticaseibacillus casei* LC-01 could not grow in media with grape seed extract concentrations above 0.25 mg/mL, whereas the growth of *L. plantarum* IFPL 935 and *L. casei* IFPL 7190 increased with increasing grape seed extract concentration [23]. Some commensal lactic acid bacteria species like *S. thermophilus* have also shown high levels of sensitivity to grape seed extract [23], thus indicating that consuming grape seed extract could potentially decrease the populations of some strains of commensal bacteria while increasing the prominence of others, thereby acting as a microbial modulator. Table 4 enumerates the responses of specific gut bacteria to grape pomace and grape pomace extracts measured in various *in vitro* studies.

3.1.1. Limitations of the existing studies assessing grape pomace prebiotic activity

While there seems to be some contrasting results in terms of which bacteria are promoted or inhibited by grape pomace, it is vital to point out that none of the studies characterized the full composition of the phenolics-enriched fractions after extraction from grape pomace and seeds. Thus, non-phenolic compounds such as abundant simple sugars or oligosaccharides could have been present in the extracts and promoted bacterial growth, acting as an easy-to-access carbon source instead of the phenolics themselves. Furthermore, none of these studies analyzed the extracts for the presence of oligosaccharides nor simple sugars nor attempted to remove the simple sugars (monosaccharides and disaccharides) by filtration or dialysis. A deeper analysis of the effects of isolated grape pomace phenolics

on commensal bacteria is therefore required to either validate or refute the claims of commensal bacteria growth on grape phenolics made by Tabasco et al. [23] and Hervert-Hernández et al. [24].

Whether grape pomace phenolics hinder or enhance bacterial growth depends largely on the ability of the specific strain to metabolize the phenolic compounds at the applied concentrations. While grape seed and pomace extracts have similar phenolic profiles, the phenolic compounds in the seed extract were over three times more concentrated than in the pomace extract studied [21]. Grape seed extract increases bacterial growth significantly more than pomace extract, whose impact on growth was found to be concentration-dependent [24]. While this could have been due to carbohydrates potentially present in the extracts, the phenolics may have played a key role in the bacteria growth. Of the phenolics tested as standards and present in the extracts, only tannic acid and catechin promoted concentration-dependent growth, while the others had no effect. Therefore, it is possible to speculate that the growth of *L. acidophilus* CECT 903, and other lactic acid bacteria in the pomace and seed extracts, was linked to their tannic acid and catechin contents [24]. Grape pomace extract also increases the growth of certain *Bacteroides*, *Enterobacteriaceae*, and *Clostridial* strains, which are thought to be the primary microbes that metabolize phenolics [34]. However, it is important to note that these do not necessarily include commensal strains of these bacteria and could include strains that have the potential to become opportunistic pathogens, as full characterization was not described in Ref. [34].

Current literature indicates that grape pomace phenolics may be able to directly, or more likely indirectly, prevent infection by inhibiting the growth of pathogens while increasing the growth of multiple commensal bacteria strains in the gut. Altering the composition of the gut microbiome to be more favorable and ingesting increased levels of phenolics, which the commensal bacteria can transform into bioactive metabolites, indicate great promise for grape pomace phenolics as a functional food product. More studies are needed, however, to determine the prebiotic effects and metabolism of highly purified grape pomace phenolics to verify their health benefits.

It has been suggested that the many health benefits linked to polyphenols intake, such as decreased blood pressure and cholesterol, could be a result of the interactions between polyphenols and gut bacteria [49]. Although it is clear that polyphenols and their metabolites promote gut health, the information on the interactions between polyphenols and the gut microbiota and their effects in humans still need to be investigated [35], since most studies on the interactions and metabolic pathways of polyphenols have been performed in animal or *in vitro* colonic models and the mechanisms are poorly elucidated [50]. Polyphenols can modify the gut microbiota composition both in terms of quantity and quality; however, it is not clear whether the increases in bacteria are impacted by increases in metabolites or the parent compounds alone [50]. Moreover, the mechanism has not been understood well due to high inter-individual variability and the absence of appropriate reference standards for the identification and quantification of phenolic metabolites [37].

3.2. The effect of grape pomace oligosaccharides on microbiota

Previous studies indicate that grape pomace has the ability to decrease pathogenic bacteria growth and increase the levels of commensal gut bacteria (Tables 3 and 4), which could be partially due to the presence of oligosaccharides, which can act as a selective carbon source. Costa et al. [45] found that an enzymatic grape pomace extract exhibited antimicrobial activity towards multiple pathogens (including *E. coli* ATCC 25922, *P. aeruginosa* ATCC 10145, *S. aureus* ATCC 25923, and *S. aureus* CCUG 60578) and attributed the effect to a combination of the xylo-oligosaccharides, phenolics, and other compounds present in the extract (Table 3). Noteworthy, the antimicrobial capacity was dramatically reduced upon gastrointestinal digestion. Furthermore, beneficial gut bacteria found in human feces like *L. acidophilus* NRRL B-1910, *L. plantarum* NRRL B-4495, and *B. bifidum* ATCC 15696 can ferment naturally occurring plant fructo-oligosaccharides and arabinogalactans similar to those found in grape pomace [51,52]. Oligosaccharides discovered in red grape seeds also improve the growth of probiotic *L. acidophilus* P18806 when used in small concentrations [29]. Additionally, an enzymatically produced grape pomace extract stimulated the growth of commensal *B. animalis* sp. *lactis* Bb12, *B. animalis* Bo, *B. longum* BG3, *L. casei* 01, and *L. rhamnosus* R11 potentially because of the xylo-oligosaccharides present in the extract that were resistant to gastrointestinal digestion (Table 4) [45].

Utilizing oligosaccharides as prebiotics to beneficially alter the gut microbiome has great potential systemic effects beyond the gastrointestinal tract. Supplementing high-fat diets with short-chain fructo-oligosaccharides increases bifidobacteria and *Clostridium coccoides* abundance and decreases *Clostridium leptum* in the mouse gut microbiome. These alterations to the gut microflora induced great metabolic changes and eliminated total weight and fat mass gain in the mice, indicating that the fructo-oligosaccharides have anti-obesity effects [53]. Other studies have also shown that oligosaccharides have anti-diabetic, anti-cancer, and cardio-protective benefits [54–56].

Bordiga et al. [30] assessed the potential prebiotic activity toward *L. plantarum* (P17630) and *L. acidophilus* (P18806) of different oligosaccharidic fractions obtained before and after distillation of grape seed pomace and they claimed that oligosaccharidic fractions improved the growth of *L. acidophilus* (P18806) during *in vitro* fermentation. Silva et al. [57] evaluated the stimulatory effects of a flour produced from Isabel grape by-products (peels and seeds) on the growth and metabolism of different probiotic strains and bacterial populations of the intestinal microbiota during an *in vitro* colonic fermentation. The by-product flour showed stimulatory effects on probiotics causing increased viable counts, decreased pH values, and stimulated the increase of the population of beneficial microorganisms, accompanied by the production of lactic acid and short-chain fatty acids. Moreover, Gil-Sánchez et al. [58] used four different grape pomace extracts rich in phenolic compounds and dietary fiber in their study. They reported that when the extracts were subjected to fermentation by fecal microbiota, the concentration of phenolic metabolites in the grape pomace extracts was increased during fermentation by microbiota in comparison to the incubation of the fecal microbiota on their own. Furthermore, the extracts tended to increase the growth of intestinal microbiota, however, it was only important for the *Enterococcus* group.

4. Culturing pathogenic bacteria, commensal bacteria, and colonic epithelial cells (Caco-2) on grape pomace and other ingredients

In addition to testing the ability of commensal bacteria to grow when exposed to grape phenolic compounds and oligosaccharides, studies have begun analyzing the effects of the pomace as well as pomace combined with resistant starch on the growth of commensal bacteria. *L. acidophilus* ATCC 43121 was able to grow in a medium containing low concentrations of grape pomace phenolic compound extracts. However, its growth improved significantly when resistant starch was added. Meanwhile, the growth of *Bifidobacteria* spp. ATCC 29521 increased at every concentration of grape pomace extract, both with and without added resistant starch (Table 4) [48]. However, this study is limited by the choice of appropriate growth media (LB), cultivation conditions, and only using an end-point optical density measurement for bifidobacteria and *Lactobacillus* strains, whereby it is difficult to interpret optimal growth outcomes and selectivity of a prebiotic component. In a separate study by Campanella et al. [33], commensal *L. plantarum* 12A, *L. plantarum* PU1, *L. paracasei* 14A, and *B. breve* 15A were able to grow on media made with whole red grape pomace (Table 4). However, these results have the caveats that the basal media in this particular study may not be free of monomeric sugars, and that the higher cell densities and increased acidification depended on the addition of 1 % glucose to the growth media, thus making it difficult to interpret the selective prebiotic activity of specific oligosaccharides present within grape pomace [33]. In any event, all these bacteria fermented the grape pomace to produce lactic acid, titratable acids, and volatile acids, and consumed all the carbohydrates within the medium. These results were particularly dramatic for *L. plantarum* PU1 and *B. breve* 15A. Each of the four tested strains also decreased the concentrations of free amino acids, gallic acid, (−)-epicatechin, and syringic acid, but the bacterial fermentation of grape pomace did not significantly alter its antioxidant activity [33]. The ability of grape pomace to inhibit linoleic oxidation increased with fermentation, particularly with *L. plantarum* PU1. Additionally, all four fermenting bacteria strains analyzed consumed all the citric acid and glycerol present in the medium, suggesting that these bacteria are able to use the pomace, including phenolic compounds such as gallic acid, as an alternative carbon source for metabolism instead of sugars [33].

These studies demonstrate that it is plausible that grape pomace could be considered a prebiotic ingredient since it can enhance the

Table 5
The responses of some gut bacteria to grape pomace and grape pomace extracts measured in various small animal studies (N/A: Not available, RGP: Red Grape Pomace, RGS: Red Grape Seed, TPC: Total Phenolic Content, WGS: White Grape Seed).

Bacteria	Growth Effect	Pomace Intervention	Study Type	Measured Carbohydrates	Measured Phenolics	Source
Adlercreutzia	Increased	WGS	Mice gut microbiome	N/A	N/A	[61]
Akkermansia	Increased	RGP extract, RGS extract	Mice gut microbiome	N/A	Extract individual phenolics	[62]
Allobaculum	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Alloprevotella	Increased	RGP extract, RGS extract	Mice gut microbiome	N/A	Extract individual phenolics	[62]
Anaerosporobacter	Increased	WGS	Mice gut microbiome	N/A	N/A	[61]
Anaerotruncus	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Blautia	Increased	WGS	Mice gut microbiome	N/A	N/A	[61]
Campylobacter jejuni	No effect	RGP	Lamb gut microbiome	N/A	TPC of diet	[63]
C. jejuni	Decreased	RGP	Piglet gut microbiome	Crude fiber of diet	TPC of diet	[64]
Clostridia	No effect	RGP	Lamb and piglet gut microbiomes	N/A, crude fiber of diet	TPC of diet	[63, 64]
Clostridium	Increased	RGP extract	Rat gut microbiome	N/A	Extract TPC and individual phenolics	[60]
Clostridium	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Escherichia coli	Decreased	RGP	Lamb and piglet gut microbiomes	N/A, crude fiber of diet	TPC of diet	[63, 64]
E. coli	Decreased	RGS	Broiler chick ileum	N/A	TPC of diet	[65]
Enterobacteriaceae	Decreased	RGP	Lamb and piglet gut microbiomes	N/A, crude fiber of diet	TPC of diet	[63, 64]
Enterococcus	No effect	RGP extract	Rat gut microbiome	N/A	Extract TPC and individual phenolics	[60]
Enterococcus	Increased	WGS	Mice gut microbiome	N/A	N/A	[61]
Enterorhabdus	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Flavonifractor	Increased	WGS	Mice gut microbiome	N/A	N/A	[61]
Odoribacter	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Oscillibacter	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Parabacteroides	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Peptococcus	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Prevotella	Decreased	RGP extract	Mice gut microbiome	N/A	Extract individual phenolics	[62]
Prevotella	Increased	RGS extract	Mice gut microbiome	N/A	Extract individual phenolics	[62]
Roseburia	Increased	WGS	Mice gut microbiome	N/A	N/A	[61]
Streptococcus	Decreased	RGP extract, RGS extract	Mice gut microbiome	N/A	Extract individual phenolics	[62]
Streptococcus	Decreased	RGS	Broiler chick ileum	N/A	TPC of diet	[65]

growth of commensal gut bacteria by acting as a food source for these bacteria; however, confirmatory studies with appropriate media and test controls, including basal media with the complete absence of carbohydrates will be required to unequivocally provide evidence of selective prebiotic activity. In the same context, *in vitro* studies confirming the inability of grape pomace to serve as a carbohydrate source for pathogen growth are necessary to assess grape pomace's prebiotic functionality. However, the studies demonstrating pathogen reduction from the abundant grape pomace phenolic compounds indicate that grape pomace likely hinders pathogen growth.

Beyond acting as a potential prebiotic, the benefit of consuming grape pomace on human health could be further increased by administering pomace in conjunction with probiotics. When combined with probiotics, low pomace concentrations decreased oxidative damage to Caco-2 cells (human epithelial colorectal adenocarcinoma cells), thus improving the antioxidant protection of the cells [33]. Another study combined grape pomace extract with *L. rhamnosus* IBNA02, *L. paracasei* 13239, and *L. acidophilus* 11692 and found the pomace extract protected the *lactobacilli* strains. This prebiotic/probiotic combination also decreased the lipopolysaccharide (LPS) O-antigen instigated inflammation of Caco-2 intestinal cells, down-regulating most inflammatory cytokine genes, proteins, and signaling molecules that were activated by LPS. This indicates the grape pomace and *lactobacilli* pre/probiotic combination has the potential to act as a treatment for intestinal inflammation [59]. More studies should be done combining grape pomace with probiotics to determine their potential health benefits, as this could be a highly effective method to valorize grape pomace.

5. Evaluating the health effects of grape pomace through small animal studies, livestock and human clinical trials

5.1. Small animal studies

The benefits of grape pomace have the potential to positively impact human health as a functional food ingredient. Initial studies of the effects of grape pomace and grape seed phenolics on pathogenic and commensal bacteria suggest that grape pomace and its seeds could positively impact human health. Small animal studies of rats and mice ingesting significant quantities of grape pomace in its various forms have been performed as the precursor to human trials to determine the impact of pomace consumption.

In a study by Chacar et al. [60], rats were given red grape pomace extract, which positively altered their gut microbiomes. Pathogenic *Clostridium* bacteria growth was inhibited by the pomace treatment, an observation that can have translational relevance in modern medical foods with the ability to reduce Clostridia (Table 5). Meanwhile, the pomace promoted beneficial bacteria with a 21–27 % increase of *Bifidobacterium* bacteria with pomace extract consumption (Table 6). The abundance of *Bifidobacterium* plateaued with high pomace extract doses, likely because catechin reached its saturation limits within the plasma of the rats. Meanwhile, commensal *Enterococcus* bacteria were unaffected by the pomace. *Lactobacillus* spp. growth decreased with grape pomace supplementation (Table 6), however, data from other studies suggest a higher pomace dose than was delivered in this study is likely to remedy this.

Seo et al. [61] used a mice model of obesity and, after supplementing a high-fat diet with chardonnay seeds, observed dramatic overall health improvements correlated with improved gut microbiome health from the chardonnay seed intervention [61]. The mice fed grape seeds had decreased body, liver, and adipose tissue weights and lower LDL levels despite having increased food intake. The abundances of *Lactobacillus* and *Bifidobacterium*, two classes well known for containing commensal and prebiotic bacteria, were found to be dependent on the flavonoid presence in the mice feces (Table 6). *Akkermansia* abundance increased with grape pomace and seed extract supplementation in mice (Table 5) and, in another study, was linked to improved gut barrier function and mucus thickness as well as decreased fat mass, insulin resistance, endotoxemia, and adipose tissue inflammation, thus helping to decrease obesity and diabetes in obese mice [61]. Meanwhile, the abundance of Firmicutes bacteria, including *Clostridium*, *Rosebuira*, *Lactobacillus*, *Enterococcus*, and *Oscillibacter* decreased in the mice feces (Table 5) [61]. *Lactobacillus* and *Oscillibacter* abundance are correlated with weight gain as they can ferment polysaccharides, releasing additional energy that can be absorbed, thus their decreased abundance likely contributed to the weight loss observed in the mice. *Roseburia*, *Adlercreutzia*, and *Enteroccoccus* are also correlated with body and adipose tissue weight gain. For example, *Aldercreutzia* was able to biotransform epigallocatechin, a phenolic compound present in grape pomace [22,61]. Therefore, the decreased concentrations of these bacteria within the mice feces further indicate that the influence of chardonnay seeds on the gut microbiome contributed to weight loss in mice.

Chardonnay seed supplementation increased Bacteroidetes and Proteobacteria within the mice feces, which created additional health benefits. *Lactobacillus* spp. and *Bifidobacterium* spp. abundance was found to be dependent on the flavonoid presence in mice feces. When catechin, epicatechin, and their oligomers were analyzed within the mice feces, their decreased concentrations indicate

Table 6
The responses of select commensal gut bacteria to grape pomace and grape pomace extracts measured in various small animal studies (N/A: Not Available, RGP: Red grape pomace, RGS: Red grape seed, TPC: Total Phenolic Content, WGS: White grape seed).

Bacteria	Growth Effect	Pomace Intervention	Study Type	Measured Carbohydrates	Measured Phenolics	Source
Bifidobacterium	Increased	RGP extract, RGP	Rat gut microbiome, lamb and piglet gut microbiomes	N/A, crude fiber of diet	Extract TPC and individual phenolics, TPC of diet	[60,63, 64]
Bifidobacterium	Decreased	WGS	Mice gut microbiome	N/A	N/A	[61]
Lactobacillus	Decreased	RGP extract, WGS	Rat gut microbiome, mice gut microbiome	N/A	Extract TPC and individual phenolics, N/A	[60,61]
Lactobacillus	Increased	RGS	Broiler chick ileum	N/A	TPC of diet	[65]

that the bacteria within the gut microbiome broke the oligomers into their metabolites and produced short-chain fatty acids, which are known to be beneficial for human health [61]. *Akkermansia* abundance also increased with grape pomace and seed extract supplementation in mice (Table 5) and was linked to improved gut barrier function and mucus thickness as well as decreased fat mass, insulin resistance, endotoxemia, and adipose tissue inflammation, thus helping to decrease obesity and diabetes in obese mice [62,66]. Chardonnay seeds, grape pomace extract, and grape seed extracts improved gut microbiomes in mice, which resulted in numerous health benefits.

In addition to having the potential to improve the gut microbiome of healthy individuals and decrease obesity-related health problems, grape pomace has the potential to help regenerate healthy gut microbiomes that have been hindered by antibiotics. Antibiotic treatment of mice decreased the diversity and abundance of microbes in their feces, particularly regarding commensal bacteria. The gut bacteria repopulate following antibiotic treatment and can develop into unhealthy gut microbiomes if insufficient commensal bacteria are present, resulting in dysbiosis. Consuming grape pomace and seed extracts following antibiotic treatment increased both the diversity and abundance of commensal microbiota within mice intestines [62]. Compared to control mice whose gut microbiomes repopulated without intervention, mice given grape pomace and seed extracts showed the grape pomace and seed extract supplementation decreased the abundance of pathogenic *Streptococcus* and *Actinobacteria* and increased commensal *Verrucomicrobia*, *Akkermansia*, and *Alloprevotella* within their feces (Table 5). The seed extract supplementation also produced increased levels of *Prevotella* (Table 5) [62]. Based on these results, grape pomace and grape seeds have the potential to act as therapeutics following antibiotic intervention to help repopulate the gut with a healthy microbiome, favoring commensal bacteria and reducing pathogenic bacteria.

5.2. Livestock studies

Grape pomace is widely used as animal feed in wine regions to bring minimal value to this abundant waste material. However, in addition to acting as an economical feed source, recent studies indicate that grape pomace diet supplementation increases livestock health and meat quality. Incorporating red grape pomace as 9 % of feed solids greatly improved both lamb and piglet gut microbiomes, and in both cases, an increase in the abundance of commensal *Bifidobacterium* and a decrease in the abundance of *E. coli* and Enterobacteriacae, a family containing many pathogens, was observed [63,64]. Grape pomace had no effect on lactic acid bacteria, *Campylobacter* spp. or *Clostridia* spp. populations in lambs but increased lactic acid bacteria growth and decreased *Campylobacter jejuni* growth in piglets [63,64]. Meanwhile, supplementing the diet of broiler chicks with 2 % grape seeds has been shown to reduce the abundance of *E. coli* and *Streptococcus* while increasing beneficial bacteria growth, including *Lactobacilli* in the ileum of chicks [65]. These results indicate that grape pomace can improve the gut microbiomes of animals, thus increasing the abundance of commensal bacteria and reducing the growth of pathogenic bacteria. Results from these studies also demonstrate improvement in gut barrier function, which has been tied to the fructan, polysaccharide, and phenolic compounds present in the grape pomace [63,64].

Grape pomace proves to be a valuable potential feed additive for drastically different livestock, as illustrated by ruminants (lambs), nonruminants (pigs), and poultry (chicks). These feeding studies indicate that grape pomace increased animal welfare by improving their health, particularly regarding their gut microbiomes. With further research with positive results, grape pomace should be increasingly utilized and valued for its ability to improve livestock health and quality. Utilizing grape pomace as a livestock health supplement would likely be a relatively quick method for grape pomace valorization as livestock already safely consume grape pomace. As grape pomace becomes better known as a valuable functional food ingredient for improving livestock products as compared to its current low value as a waste, its market value can increase. This can make livestock feed supplementation a potentially viable valorization strategy for grape pomace.

5.3. Human trials

Small animal studies were followed by human clinical trials to examine the cardiovascular health effects of chardonnay seeds and, particularly, chardonnay seed phenolic compounds. Initial trials and the primary study found chardonnay seed supplements to be safe in low doses of 4.8 g/day but to cause non-severe negative gastrointestinal effects in higher doses of 24 g/day [67]. Interestingly, consuming phenolics-rich or phenolics-free chardonnay seed supplements had similar effects on the health of the participants, and both groups experienced similar levels of endothelial function improvement and peripheral endothelial function, including decreased systolic and diastolic blood pressure [67]. Corban et al. [67] speculated that phenolics, which have largely been assumed to cause the health benefits, were not the impactful compound in chardonnay seeds, and dietary fiber was responsible for the observed cardiovascular health benefits as dietary fibers have previously demonstrated cardioprotective effects.

Costabile et al. [68] investigated the effects of the consumption of a drink from red grape pomace on glucose/insulin and triglyceride responses in twelve healthy men (aged 20–40 years) and the relationship between plasma levels of phenolic metabolites and metabolic parameters. The concentrations of glucose, insulin, triglyceride and phenolic metabolites were measured at fasting, 3 h after drinking 250 mL of a red grape pomace drink rich in polyphenols (1562 g of total polyphenols as gallic acid equivalents including anthocyanins (70 %), followed by flavan-3-ol monomers (23 %)), over 5 h after the standard meal and at fasting on the next day. They reported that the consumption of the experimental drink rich in polyphenols decreased post-meal insulin response and improved insulin sensitivity, probably due to the increase in gallic acid levels [68]. Moreover, in their previous publication [37], they found that the intake of the same experimental drink increased the plasma levels of several phenolic metabolites, however, there was a high inter-individual variability in the urine and plasma samples of ten volunteers.

Gil-Sánchez et al. [38] researched the impact of grape pomace supplementation on different biochemical and molecular biomarkers

as well as in the composition and activity of the human gut microbiota in 10 healthy women (age ranged from 25 to 65 years). The participants took two capsules per day of the red grape pomace extract (1.4 g of extract/day with the average daily composition of 923.58 mg and 54.42 mg fiber and phenolics, respectively) for 21 days after an initial washout period of 10 days. An important decrease in blood fasting glucose levels and significant changes in the short-chain and medium-chain fatty acid profiles were observed after the supplementation. However, the supplementation did not change the content of fecal and urine phenolic metabolites [38].

Ramos-Romero et al. [69] researched the potential modifications in microbiota profiles after grape pomace supplementation. Forty-nine (aged 18–70 years) subjects at cardiometabolic risk received a daily dose of 8 g of grape pomace (dietary fiber: 68.2 %, polyphenols: 29.6 %) for 6 weeks. The levels of total bacteria and Bacteroidetes, Firmicutes, Lactobacilliales, *Bacteroides* and *Prevotella* were estimated in fecal DNA. Grape pomace supplementation decreased insulin levels only in half of the participants (responders) and increased the proportion of *Bacteroides* in non-responsive subjects. Grape pomace supplementation did not change gut microbiota profile of the whole group significantly, except for a decrease for Lactobacilliales [69].

Taladrid et al. [70] tested the consumption of a seasoning obtained from red grape pomace for the control of hypertension and glycaemia. The research was carried out on 17 high-risk cardiovascular subjects and 12 healthy subjects. The results showed that the culinary use of a grape pomace seasoning (dietary fibre: 19 mg/g and total phenolic content: 47.96 mg of gallic acid equivalents/g) as 2 g per day for 6 weeks significantly decreased blood pressure and fasting blood glucose in both healthy and high-risk cardiovascular people. Fecal metabolites did not show an important change compared to basal conditions, however, there were small differences in the microbiota of healthy and HCR subjects [70].

Additionally, Annunziata et al. [71] evaluated the effect of grape pomace extract with or without pectin on trimethylamine N-oxide (TMAO), which is recognized as a biomarker of increased cardiovascular risk and oxidized low-density lipoprotein (ox-LDL) serum levels in a group of overweight/obese subjects (18–83 years old men and women). The participants were subjected to only grape pomace extract (300 mg Taurisolo®, twice daily) or grape pomace extract + pectin (300 mg Taurisolo®+300 mg pectin, twice daily). The study included a 4-week run-in period, an 8-week intervention period and a 4-week follow-up period; and blood samples were collected after 12 h of fasting at weeks 0, 4, 8, 12, and 16. After an 8-week treatment, they observed an important reducing effect of the ox-LDL and TMAO serum levels of grape pomace extracts, but the addition of pectin did not show a value-added for the observed effect. Furthermore, after the 4-week follow-up period, the ox-LDL and TMAO serum levels did not increase significantly in both groups [71]. Martínez-Maqueda et al. [72] investigated the effect of grape pomace (including both extractable and non-extractable polyphenols) on markers of metabolic syndrome (MetS) in two different 6-week periods, separated by a 4-week wash-out. The participants with MetS (22 women, 28 men, aged 20–65) were randomly assigned to two groups (supplementation of 8 g/day and control) and half of the participants were subjected to an oral glucose tolerance test. Supplementation with grape pomace significantly improved fasting insulinemia, without affecting other cardiometabolic risk parameters [72]. Urquiaga et al. [73] examined the effect of the intake of wine grape pomace flour (WGPF) prepared from red wine grapes in 38 male subjects (control group: 13), 30–65 years of age with at least one component of metabolic syndrome. The intervention group received 20 g of WGPF (including 10 g of dietary fiber, 822 mg of polyphenols and an antioxidant capacity of 7258 ORAC units) per day for 16 weeks. The results showed that the supplementation with WGPF significantly lowered blood pressure and increased plasma concentrations of γ-tocopherol and δ-tocopherol [73]. Cao et al. [74] carried out a clinical trial about the effect of grape seed proanthocyanidin extract (GSPE) on atherosclerotic plaques and assessed the effect of GSPE on 287 patients (control group: 141) diagnosed with asymptomatic carotid plaques or abnormal plaque carotid intima-media thickness. The GSPE group received GSPE as 200 mg/day and carotid ultrasound examination was carried out at baseline and 6, 12, and 24 months during follow-up. GSPE showed atherogenic effects even after treatment of 6 months and reduced plaque thickness [74]. Zhang et al. [75] evaluated the impact of grape seed extract treatment on blood pressure by meta-analyzing available randomized controlled trials. The results indicated that grape seed extract played an important effect on blood pressure, and this was more evident in young or obese subjects and patients with metabolic disorders [75].

More investigation is needed to determine the impact of naturally occurring grape oligosaccharides, phenolics, and whole grape pomace on human health.

6. Conclusion

Overall, microbial analyses showed that the compounds within the pomace matrix largely prevent pathogen proliferation while stimulating commensal bacteria growth in cell cultures and small animal studies, thus leading to diverse gut microbiota composition and resulting in improved intestinal health and function. Grape pomace also protects probiotic strains from the harsh conditions of the upper GI tract, enabling them to reach the colon, thus indicating the tremendous potential of grape pomace in a prebiotic/probiotic combination supplement to improve human gut health. Considering the impressive results observed in animal models, additional human studies should be performed to evaluate additional grape pomace applications, such as delivering synergistic combinations with commercially available probiotic strains that would likely be able to grow on the prebiotics naturally found in grape pomace. Well-designed clinical studies utilizing chemically defined and purified fractions of grape pomace will enable the differentiation of the contributions of phenolics and oligosaccharides.

Declarations

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Declaration of competing interest

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

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