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Investigating the effect of bioactive compounds in New Zealand-grown Damson plum on the human gut microbiome.

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Abstract

The gut microbiome can be strongly influenced by dietary intake, particularly fruits and vegetables that are rich in bioactive compounds. Polyphenols may influence microbial community composition and metabolic activity, potentially contributing to health benefits beyond their nutritional value. Damson plums are rich in polyphenols, particularly in their skin, which may influence gut microbial composition and function. This study tested whether damson plum substrates altered microbial community composition and metabolite production compared with a potato starch control, and whether the inclusion of damson skin had additional effects, using an in vitro digestion and fermentation model. Pomegranate was included as an additional substrate comparator. SCFA production following fermentation was quantified using gas chromatography (GC), and DNA was extracted for shotgun metagenomic sequencing to characterise microbial community composition. Alpha diversity indices were significantly higher in both damson treatments than in the potato starch control but did not differ between the damson plum treatments ($p = 0.206$), whereas pairwise PERMANOVA indicated significant differences in microbial composition between damson flesh and skin-on treatments (BH-adjusted $p = 0.0072$), with higher abundance of *Lachnospira*, *Vescimonas*, and *Escherichia* in the skin-on treatment. Compared with the potato starch control, both damson treatments produced significantly higher butyrate and propionate concentrations and lower acetate concentrations. SCFAs production in the treatments was consistent with polyphenol-rich substrates, with higher butyrate and propionate production. Functional profiling revealed lower relative abundances of KEGG orthologs associated with central metabolic functions in the damson plum treatments compared with pomegranate. In contrast, orthologs associated with aromatic metabolism were increased in damson plum relative to the potato starch. These findings suggest that damson plum flesh and skin-on treatments were associated with changes in gut microbial communities and functional profiles and SCFA production compared with the

potato starch control. However, although microbial community composition differed between the damson plum treatments, their SCFA profiles were broadly similar, suggesting that the inclusion of skin had a greater influence on microbial community composition than on fermentation end products.

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List of abbreviation

Abbreviation	Definition
BH	Benjamini–Hochberg
KEGG	Kyoto Encyclopedia of Genes and Genomes
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
SCFA	Short-chain fatty acid
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SSF	Simulated Salivary Fluid
TDF	Total Dietary Fibre

Chapter 1. Introduction

Recent studies have demonstrated that the human gut microbiome plays a critical role in physiological, immune, metabolic, and neurological processes (Brooks et al., 2023; Kang & Martin, 2017; Riquelme et al., 2019). The composition of the gut microbiome is influenced by multiple factors, such as age, diet, drugs, health conditions, and geography, shape the gut microbiome (Borgonovi et al., 2022). The human gastrointestinal tract is a complex ecosystem that harbours the highest abundance of bacteria in the human body. The large intestine contains approximately 100 billion bacterial cells/mL of intestinal content, whereas the lower small intestine (Ileum) contains approximately 100 million bacterial cells/mL (Sender et al., 2016). Owing to its extensive metabolic capacity, the gut microbiota is often referred to as a hidden metabolic organ that contributes to nutrient metabolism, production of bioactive metabolites and host physiological regulation (Attri et al., 2022). The dominant bacterial phyla found in the healthy gut microbiome include Actinomycetota, Bacteroidota, Bacillota, Pseudomonadota, and Verrucomicrobia (listed in alphabetical order). In a healthy state, these gut microorganisms form a symbiotic relationship with the host, regulating nutrient metabolism, defending against pathogens, and modulating immune signalling pathways to support host health and homeostasis (Afzaal et al., 2022).

Diet is one of the major factors shaping the composition and function of the human gut microbiome. Dietary patterns characterised by high intakes of energy-dense, nutrient-poor foods and low consumption of fibre-rich foods have been associated with reduced microbial diversity and alterations in microbial composition, a condition commonly referred to as dysbiosis. Dysbiosis has been associated with obesity, cardiovascular disease, inflammatory bowel disease, and type 2 diabetes (Bohara et al., 2021). Consequently, increasing consumption of fibre-rich fruits and vegetables has been proposed as a strategy to support microbial diversity and gut health. Fruits and vegetables are important sources of dietary fibre, vitamins, and

phytochemicals that support both human health and gut microbial diversity. However, despite strong evidence for their health benefits, achieving the recommended daily intake of at least 400 g of fruits and vegetables remains challenging in many countries (Pem & Jeewon, 2015). Plant-derived bioactive compounds are naturally occurring compounds present in plants that exert biological effects in humans and other organisms. These compounds exhibit antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties (Alvarez-Leite, 2025). Many plant-derived bioactive compounds can influence microbial community composition associated with host health (Chen et al., 2022), including the production of metabolites such as SCFAs (Portincasa et al., 2022).

Damson plum (*Prunus domestica spp. insititia*) is a type of European plum containing numerous minerals, antioxidant properties, vitamins, phenolic compounds, and dietary fibre, some of which fall under the category of bioactive compounds. Damson plums grown in New Zealand (e.g. Hawke's Bay) are ovoid and small with dark purple skin. Damson plums contain dietary fibre, vitamins, minerals, and a range of antioxidant phytochemicals. Studies have shown that damson plums are rich in bioactive compounds, including anthocyanins, flavonoids and phenolic acids. These compounds have been associated with anti-inflammatory, and antioxidant activities, and may contribute to gastrointestinal health and other physiological benefits (Rashidinejad & Ahmmed, 2024; Wallace, 2017; P. Xia et al., 2023).

Despite the nutritional and phytochemical composition of damson plums, relatively few studies have investigated New Zealand-grown cultivars and their effects on the gut microbiome. Furthermore, the role of microbial biotransformation in mediating the biological activity of damson plum polyphenols remains poorly understood. Based on previous reports that plum skin contains high concentrations of polyphenolic compounds, this study hypothesised that damson plum preparations with intact skin would differentially influence gut microbial community composition and metabolic activity compared with peeled fruit. To test this

hypothesis, an in vitro gastrointestinal digestion and faecal fermentation model inoculated with pooled adult faecal microbiota was used to simulate the human gastrointestinal environment. Changes in microbial community composition were assessed using shotgun metagenomic sequencing, while microbial metabolic activity was evaluated through SCFA quantification.

Chapter 2. Literature review

2.1 Abstract

There has been a strong focus on researching the human gastrointestinal tract microbiome as new technologies (Next-generation sequencing) unlock a deeper understanding of gut microbiota composition. One way to modulate the gut microbiota is through interactions with bioactive compounds through consuming food, especially vegetables and fruits. Bioactive compounds can exert health benefits and directly influence composition, or act through processes such as metabolism and fermentation, which often produce metabolites like short-chain fatty acids. Butyrate, acetate, and propionate are the known metabolites that can influence host health. Numerous studies have revealed that gut microbiome composition directly regulates overall human functions. Metabolic diseases such as type 2 diabetes, irritable bowel syndrome, polycystic ovary syndrome and obesity are strongly associated with specific microbiome signatures. Fruit contains compounds that promote beneficial bacteria and decrease non-beneficial bacteria in the human large intestine (colon) and small intestine, where the interactions between nutrients and gut microbiota occur. The plum is a type of stone fruit and is commercially available across many countries. There are several ways to enjoy plums: fresh, dried, stewed, pickled and even in alcohol. The fruit is loved by many for its high nutritional value and flavour. European plums (*Prunus domestica L.*) and Japanese plums (*Prunus salicina Lindl*) are the most commonly eaten cultivars. Damson plum (*Prunus insititia*) is categorised under *Prunus domestica* ssp. and has yellow flesh with dark blue skin. This plum cultivar is grown worldwide, but in New Zealand, it is primarily cultivated in Hawke's Bay, where the climate is hot and dry in summer and mild in winter.

This literature review will discuss the potential nutritional values of New Zealand-grown damson plums *(Prunus insititia)* for human consumption and the correlation between gut microbiome composition and bioactive compounds.

2.2 Introduction

Interest in the gut has continued to increase, and many studies have been conducted since the Human Microbiome Project (HMP) was launched in 2007 (Blum, 2017; Human Microbiome Project Consortium, 2012). Advances in sequencing technologies and reductions in costs have accelerated research into the human microbiome; however, the host-microbe interaction is still not fully understood (Cullen et al., 2020). The gut microbiota is one of the most diverse microbial communities in the human body, consisting of trillions of microorganisms that live symbiotically in the gut and contribute to nutrient metabolism, enhance gut barrier functions, and modulate immune response (Attri et al., 2022). High-throughput sequence technologies, particularly next-generation sequencing (NGS), have greatly expanded our understanding of the gut microbiome and enabled the identification of previously uncharacterised microbial taxa within the human gastrointestinal tract. (Panek et al., 2018). Alterations in gut microbiome composition have been associated with obesity, which often leads to other life-threatening diseases such as cardiovascular disease, type 2 diabetes, and cancer (Rashidinejad & Ahmmed, 2024; C. Xia et al., 2023). Multiple factors impact the gut microbiota composition, including childbirth, antibiotics, diet, and age (Brooks et al., 2023; Lathakumari et al., 2024; Singh et al., 2017). Among these factors, daily dietary intake significantly shapes the composition and diversity of the gut microbiome. There is clear evidence that animal-based and plant-based diets are associated with distinct gut microbial communities (David et al., 2014). Food undergoes digestion in the gastrointestinal tract with most proteins, fats, and carbohydrates being digested and are absorbed in the small intestine. In contrast many dietary polyphenols, fibres, and other non-digestible compounds escape digestion and reach the colon, where they are metabolised by the gut microbiota. Through this process, gut microorganisms transform dietary substrates into a range of metabolites, including short-chain fatty acids and phenolic metabolites, which can influence both microbial ecology

and host physiology (Dominguez-Avila et al., 2021; Hall, 2016). Consequently, the interaction between dietary components and microbial metabolism is increasingly recognised as an important determinant of human health.

Fruits and vegetables are vital components of a healthy diet, providing essential nutrients and bioactive compounds that, although not considered essential nutrients for humans, exert beneficial effects on human health (El-Saadony et al., 2025). Among the bioactive compounds present in plants, polyphenols are one of the most widely distributed classes of phytochemicals and are widely studied (Pandey & Rizvi, 2009). Although a proportion of dietary polyphenols are absorbed in the small intestine, many reach the colon where they undergo extensive microbial biotransformation, generating metabolites that may influence both microbial community structure and host health.

Stone fruits such as apricot, cherry, peach, plum and nectarine are rich in polyphenols (Redondo et al., 2017). Plums are recognised as a rich source of dietary fibre and polyphenolic compounds. These bioactive compounds have been associated with antioxidant, anti-inflammatory, and gut microbiota-modulating properties. Among stone fruits, plums, particularly the New Zealand-grown damson plums (*Prunus insititia*), have received relatively little research attention despite their potential health benefits (Rashidinejad, 2024; C. Xia et al., 2023).

Although the effects of polyphenol-rich foods and several plum varieties on the gut microbiota have been investigated, limited research has focused specifically on New Zealand damson plums. Damson plums contain dietary fibre and polyphenolic compounds that may reach the colon and undergo microbial biotransformation, potentially influencing microbial community composition and metabolic activity. However, the effects of these bioactive compounds on gut microbiome composition and function remain poorly understood. This literature review examines the current evidence regarding the bioactive composition of damson plums and evaluates their potential impact on the human gut microbiota.

2.3 Background and nutritional value of damson plum

2.3.1 Plums in New Zealand

Plums have been discovered approximately 2,000 years ago and have been commonly consumed in various forms, including fresh, jams, pickles, prunes, and chutneys (Igwe & Charlton, 2016). The genus *Prunus*, belonging to the Rosaceae family, includes almonds, plums, peaches, cherries, apricots, nectarines, and berries, and is known to comprise over 200 species (Gharaghani & Solhjoo, 2021). Those are recognised as "stone fruit" or "summer fruit" in New Zealand. The most common commercial species are European plums (*Prunus domestica L.*) and Japanese plums (*Prunus salicina Lindl*). The damson plum (*Prunus insititia*) is a subspecies of *Prunus domestica*. Originally from Europe, it has dark blue skin and yellow flesh. It is unclear when damson plums were introduced to New Zealand, but they are mainly grown in the Hawke's Bay region. They are smaller than regular plums and taste tangy, most likely due to their polyphenolic content (Afanador-Barajas et al., 2022; Igwe & Charlton, 2016).

2.3.2 Nutritional values in Plum

In addition to bioactive compounds, plums provide vitamins and minerals that contribute to human health (Rashidinejad, 2024; P. Xia et al., 2023). The contents of bioactive compounds can be influenced by cultivar, processing conditions, and age. For example, research conducted by Muzzaffar et al. showed that anthocyanins degrade after drying and continue to decrease over time. In addition, there was a significant reduction in β-carotene levels in the specific plum species during the first three months of storage (Muzzaffar et al., 2024). Phenolic compounds extracted from plums have demonstrated antibacterial properties, inhibiting the growth of *Staphylococcus epidermidis*, *Proteus mirabilis*, and *Staphylococcus aureus* due to its phenolics (Sanchi Mehta et al., 2014). Phenolic compounds are among the most abundant bioactive compounds found in plums, and more phenolic compounds were measured in plum skin. The phenolic content in skin ranged from 249.91 ± 6.85 to 773.31 ± 12.66 mg of gallic acid

equivalents per 100g, with Alutus plums showing the highest antioxidant activity among 12 other cultivars (Rop et al., 2009).

Polyphenols have a positive impact on human physiological function due to their antioxidant and anti-inflammatory properties (Sorrenti et al., 2023). Anthocyanins are also known to provide physiological benefits such as anti-obesity, antimicrobial and antitumour activities (Smeriglio et al., 2016). The research by Liaudanskas et al. showed that the amount of quinic acid surpassed the other phenolic compounds in some plum cultivars. Quinic acid is a type of organic acid and bioactive (Liaudanskas et al., 2020). Quinic acid has been reported to possess anti-inflammatory and other bioactive properties, enhance DNA repair, and is inhibitory for nuclear factor-kappa B (NF-kB). NF-κB is a key regulator of inflammatory signalling pathways and controls the expression of inflammatory cytokines, which act as signalling molecules that coordinate immune responses. NF-kB activation is specifically beneficial for patients with inflammatory bowel disease (S. Li et al., 2025).

Plum seeds are usually discarded as waste, but they are rich in proteins and lipids and have been considered an alternative source of bioactive compounds (Savic & Savic Gajic, 2020).

2.3.3 Processing of plums for consumption

Drying plums to produce prunes extends shelf life and concentrates many nutrients, allowing year-round availability. However, some phenols can be destroyed during the drying process. Ascorbic acid and anthocyanins, for example, are sensitive to heat (González-García et al., 2014; Muzzaffar et al., 2024; Vinson et al., 2005). While *Prunus domestica* has been widely researched, few studies have investigated damson plums, or how consuming these might contribute to health.

2.4 Bioactive compounds in damson plums

2.4.1 Background of bioactive compounds

Bioactive compounds are naturally occurring compounds in foods that may exert biological effects beyond their nutritional value (Biesalski et al., 2009; El-Saadony et al., 2025; Martín Ortega et al., 2019). These compounds have been associated with beneficial physiological effects and may contribute to disease prevention. Vitamins and minerals are generally classified as essential nutrients rather than bioactive compounds. Many bioactive compounds found in fruits and vegetables possess antioxidant properties and may help reduce oxidative stress by neutralising free radicals (Skrovankova et al., 2015). Oxidative stress can damage important biomolecules, including proteins, lipids, and DNA, and has been implicated in the development of numerous diseases (Sies et al., 2017). Although the bioactive composition of damson plums has not been fully characterised, several studies have identified and quantified a range of bioactive compounds in plum cultivars (Ayub et al., 2023). The concentrations of phenolic compounds, vitamin C, and β-carotene differ between plum skin and flesh, with many of these compounds occurring at higher concentrations in the skin (Gil et al., 2002).

2.4.2 Phenolic compounds

Phenolic compounds are a major class of plant bioactive compounds that have been associated with antioxidant, antibacterial, antiallergenic, and anti-inflammatory activities (Shahidi & Ambigaipalan, 2015). Structurally, phenolic compounds contain at least one aromatic ring (Figure 1) bearing one or more hydroxyl groups, and more than 8,000 plant phenolic compounds have been identified (de la Rosa et al., 2019). Phenolic compounds can be broadly classified into flavonoids and non-flavonoids. Flavonoids are the predominant phenolic compounds found in fruits, while non-flavonoid phenolics include phenolic acids, tannins, stilbenes, and lignans (Al Mamari, 2021; Albuquerque et al., 2011; de la Rosa et al., 2019). Although flavonoids are the major phenolic compounds present in most fruits, damson plums

also contain substantial concentrations of non-flavonoid phenolics, with total phenolic contents reported at approximately 375 mg gallic acid equivalents (GAE) per 100 g (Suleria et al., 2020; P. Xia et al., 2023).

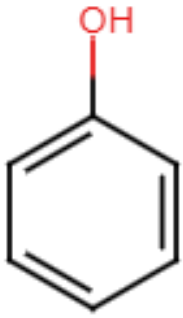


Figure 1. Basic structure of phenol.
Drawn by Chemical sketch tool.

2.4.3 Carotenoids

Carotenoids are plant pigments responsible for the yellow, orange, and red colours of many fruits and vegetables. As humans cannot synthesise carotenoids, dietary intake is required and has been associated with various health benefits, including reduced risk of age-related macular degeneration and other chronic diseases (Eggersdorfer & Wyss, 2018). Gil et al. (2002) reported carotenoid concentrations of 231 µg/100 g and 113 µg/100 g in Black Beaut and Angeleno plums, respectively (Table 1). Carotenoid content varies among plum cultivars, with green-yellow cultivars generally containing higher concentrations than purple cultivars (Deng et al., 2023). Although the carotenoid content of damson plums has not been specifically measured, their dark purple pigmentation suggests that anthocyanins may be more abundant than carotenoids.

Table 1. Total phenolics, vitamin C, and total carotenoid contents in plum cultivars. Adapted from (Gil et al., 2002).

Plum cultivar	total phenolics (mg/100g of plum)	vitamin C (mg/100 g of plum)	total carotenoides (µg/100 g of plum)
Wickson	42.0	9	87
Black Beaut	109.2	2.9	231
Red Beaut	57.4	10.2	87
Santa Rosa	54.8	2.5	83
Angeleno	82.4	4.2	113

2.4.4 Vitamins

Damson plums contain small amounts of vitamins A, C, B6 and E. Vitamin C is an essential nutrient that functions as an antioxidant and plays important roles in collagen synthesis and immune function; deficiency can result in scurvy (Carr & Maggini, 2017). Humans cannot synthesise vitamin C and therefore require regular dietary intake from fruits and vegetables (Fenech et al., 2018). Damson plums also contain vitamin A, an essential micronutrient involved in vision, growth and immune function (Huang et al., 2018). Inadequate vitamin A intake can lead to impaired immune function and visual disorders, including xerophthalmia (Debelo et al., 2017).

2.4.5 Dietary fibre

Dietary fibre consists of plant-derived carbohydrates that resist digestion by human enzymes in the upper gastrointestinal tract (Dai & Chau, 2017). Although dietary fibre and prebiotics are not synonymous, some dietary fibres can function as prebiotics by selectively stimulating the growth and activity of beneficial gut microorganisms (Gibson, 2004). In contrast, probiotics are live microorganisms that confer health benefits when consumed in adequate amounts (Hijova et al., 2019). Plums contain both soluble and insoluble fibre (Table 2). Soluble fibre, including pectin and gums, dissolves in water to form viscous gels and is readily fermented by gut microorganisms. Insoluble fibre, such as cellulose and lignin, is less readily fermented and contributes primarily to stool bulk and intestinal transit (Dai & Chau, 2017; Dhingra et al.,

2012). Through fermentation in the colon, dietary fibre can influence gut microbial composition and promote the production of metabolites such as SCFAs, which contribute to gut and host health.

Table 2. Dietary fibre content in fruits.
Adapted from (Dhingra et al., 2012).

	Dietary fibre (g/100g edible portion)		
	Total	Insoluble	Soluble
Plums	1.6	0.7	0.9
Peach	1.9	1	0.9
Grapes	1.2	0.7	0.5

2.4.6 Organic acids

Organic acids are naturally occurring compounds that contain one or more carboxyl groups and are widely distributed in foods and biological systems. In fruits, organic acids contribute to flavour, acidity, and overall quality. Plums contain several organic acids, including quinic acid, which has been identified as a bioactive constituent in some cultivars (Liaudanskas et al., 2020). Organic acids are also important products of microbial metabolism. During fermentation of dietary substrates in the colon, gut microorganisms produce short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which play important roles in gut and host health (Koh et al., 2016). Consequently, analysis of organic acids provides insight into microbial metabolic activity and the potential functional effects of dietary interventions.

2.5 Connection between the human gut microbiota and bioactive compounds

2.5.1 Human gut microbiome

The term "human gut microbiota" comprises the diverse community that inhabit the gastrointestinal tract, while the term “human gut microbiome” encompasses both these microorganisms and their collective genetic material. The human gastrointestinal tract contains trillions of microorganisms, and thousands of prokaryotic species have been identified through culture-dependent and culture-independent approaches (Kim et al., 2021). Despite extensive

research, many gut microorganisms remain uncultured or incompletely characterised, indicating that the full extent of gut microbial diversity is yet to be determined (Jensen, 2025). The gut microbiota is dominated by members of the phyla Actinomycetota (formerly Actinomycota), Bacillota (formerly Firmicutes), Bacteroidota (formerly Bacteroidetes) and Proteobacteriota (formely Proteobacteria), which together comprise the majority of bacterial taxa in the human gut.

2.5.2 Dysbiosis and the gut microbiome

Dysbiosis refers to an imbalance in the gut microbiota that is typically characterised by reduced microbial diversity, loss of beneficial microorganisms, and increased abundance of potentially harmful taxa (Hrncir, 2022). Although numerous studies have demonstrated strong associations between gut microbiota composition and host health, there is currently no universally accepted definition of a "healthy" gut microbiome due to substantial inter-individual variation (Hou et al., 2022; Liu et al., 2021). However, the dominant phyla found in the human gut microbiota are the Bacillota and Bacteroidota (Attri et al., 2022).

Diet is one of the most important factors influencing gut microbial composition (Requena et al., 2022; World Health Organization, 2025; Zhu et al., 2020). Dietary interventions can rapidly alter microbial community structure, even over relatively short time periods. For example, David et al. demonstrated that a five-day animal-based diet significantly altered gut microbial composition and increased the abundance of bile-tolerant microorganisms compared with a plant-based diet (David et al., 2014). Similarly, ketogenic diets have been shown to modify the relative abundance of major bacterial phyla, including Actinomycetota, Bacteroidota, and Bacillota (Ang et al., 2020). Dysbiosis has frequently been associated with obesity and other metabolic disorders. Alterations in microbial composition may influence energy harvest, appetite regulation, and metabolic homeostasis, potentially contributing to disease progression (Liu et al., 2021). While specific taxonomic signatures associated with obesity remain

inconsistent between studies, differences in gut microbial composition between obese and non-obese individuals have been widely reported (Duan et al., 2021). Given the significant role of diet in shaping the gut microbiota, foods rich in bioactive compounds have gained interest for their potential to mitigate dysbiosis (Chen et al., 2022).

2.5.3 Effects of fruit-derived bioactive compounds on the gut microbiome

Many plant-derived bioactive compounds, particularly dietary fibre and polyphenols, reach the colon where they are metabolised or fermented by the gut microbiota (Williamson & Clifford, 2017). Dietary bioactive compounds and the gut microbiota interact closely with one another. Studies have reported that polyphenol-rich fruits may promote the growth of beneficial microorganisms and modulate microbial metabolic functions, suggesting a potential role in maintaining gut microbial balance and reducing dysbiosis (Maqsood et al., 2025). Microbial metabolism of dietary substrates and polyphenols contributes to the production of metabolites such as SCFAs, bile acids, and amino acids. These metabolites interact with host receptors and intestinal cells, thereby influencing gut physiology and health (H. Li et al., 2025).

2.5.4 The interaction between the gut microbiota and bioactive compounds in plums

In addition to the vitamins, minerals, and carbohydrates, plums contain bioactive compounds that interact with the microbiota. Gut microorganisms can metabolise these compounds into bioactive metabolites that may influence host health. Conversely, bioactive compounds can alter the composition and activity of the gut microbiota by selectively promoting or inhibiting the growth of specific microorganisms (Chen et al., 2022). Dietary components interact extensively with the gut microbiota, which can metabolise bioactive compounds before their absorption by the host (Laparra & Sanz, 2010). Dietary fibre and polyphenols are among the major bioactive components of plums that interact with the gut microbiota. Many polyphenols escape digestion in the upper gastrointestinal tract and reach the colon, where they are metabolised by resident microorganisms. Similarly, dietary fibre serves as a substrate for

microbial fermentation, resulting in the production of SCFAs that contribute to gut and host health. In addition to fibre and polyphenols, vitamins and minerals may also influence gut microbial composition and function. The gut microbiota can enhance mineral bioavailability by producing SCFAs that lower intestinal pH and by degrading compounds that limit mineral absorption (Barone et al., 2022). Certain vitamins also interact with the gut microbiota, with some microbial taxa capable of synthesising vitamin K and several B vitamins, while dietary vitamins may influence microbial diversity and metabolic activity (Barone et al., 2022; Pham et al., 2021).

Dietary fibre

Dietary fibre can modulate the gut microbiota through microbial fermentation of fermentable fibre fractions in the colon. Dietary fibre can increase the abundance of beneficial gut microorganisms and serves as a substrate for microbial fermentation, resulting in the production of metabolites such as SCFAs (Jin et al., 2023). SCFAs act as a signalling molecule to activate pathways to trigger physiological functions such as metabolism, immune regulations and gut barrier integrity (Cronin et al., 2021 ; Ying et al., 2023). SCFAs exert their effects on the host through multiple ways, and one of which is through binding to G-protein-coupled receptors (GPCR) (Martin-Gallausiaux et al., 2021).

Polyphenols

Polyphenols are another type of bioactive compounds that are rich in plums. A small portion of polyphenols (5 – 10%) are absorbed in the small intestine, while a large proportion of them (90 – 95%) are transported to the large intestine (Loo et al., 2020; Wang et al., 2022). Polyphenols reaching the large intestine are metabolised into smaller phenolic metabolites. These metabolites, along with unmetabolized polyphenols, can modulate the gut microbiome and acts as prebiotic-like effects promoting beneficial bacteria such as *Akkermansia spp* (Dominguez-Avila et al., 2021; Rodríguez-Daza et al., 2020). This growth-promoting effect

may occur because some polyphenols can serve as carbon sources for microbial metabolism (Rodríguez-Daza et al., 2020).

Polyphenols not only have the ability to increase the growth of beneficial bacteria but also have antimicrobial properties, inhibiting the harmful group, *Escherichia coli*, *Clostridium perfringens*, and *Helicobacter pylori*. One possible mechanism is a change to the bacterial cell membrane, which can affect the growth of beneficial and harmful bacteria. These shifts in microbial balance may alter SCFA production (Plamada & Vodnar, 2021). The other mechanisms include disrupting cellular processes and preventing biofilm formation. These actions can suppress pathogenic bacteria, support gut health and contribute to maintaining microbial balance (Rodríguez-Daza et al., 2020).

A study found that in an in vivo study on mice, polyphenols from blueberries did not change the Bacillota/Bacteroidota ratio; however, an increase in beneficial bacterial species was noted, including *A. muciniphila* and *A. equolifaciens* (Dominguez-Avila et al., 2021). Another in vitro study using human faecal samples showed that polyphenols in date fruits significantly increased the abundance of bifidobacteria, a genus commonly categorised as a probiotic (Eid et al., 2014). Although the evidence suggests that polyphenols can modulate the gut microbiota, individual polyphenols may affect the microbial population differently (Kasprzak-Drozd et al., 2021; Parkar et al., 2008).

Minerals and vitamins

Damson plums contain several micronutrients including vitamin C, potassium, and phosphorus (New Zealand Food Composition Database, 2024). The gut microbiota can influence the bioavailability of dietary minerals through several mechanisms. SCFAs produced by microbiota lower intestinal pH to increase mineral solubility, while bacterial phytases break down phytic acid, releasing minerals that would otherwise remain bound and unavailable for uptake (Barone et al., 2022). Interactions between vitamins and the gut microbiota are also bidirectional. Certain gut microorganisms can synthesise vitamins, including vitamin K and

several water-soluble vitamin B contributing to host nutrient status. For example, vitamins like vitamin C has been reported to influence gut microbial diversity and SCFA production (Barone et al., 2022; Pham et al., 2021).

Short-chain fatty acids

Dietary fibres and polyphenols present in fruits may influence host health in part through their effects on microbial SCFA production. Acetate, propionate and butyrate are the major SCFAs produced during microbial fermentation of dietary fibre and play important roles in host metabolism, immune regulation and gut health (Facchin et al., 2024; Nogal et al., 2021). SCFAs serve as signalling molecules and energy sources for both the host and the gut microbiota and have been associated with a range of beneficial health outcomes. In a cohort of 948 female participants, higher circulating acetate concentrations were associated with lower visceral adiposity. The result shows that higher acetate levels were associated with lower visceral adiposity. Several bacteria genera including *Coprococcus*, *Barnesiella*, *Ruminococcus*, *Ruminococcaceae NK4A2* were positively associated with acetate production while *Lachnoclostridium* and *Bacteroides* were negatively associated (Nogal et al., 2021).

2.6 Methodologies of the gut microbiota study

Next-generation sequencing (NGS) has become the most widely used approach for studying the human gut microbiota. NGS enables high-throughput sequencing of microbial nucleic acids allowing the identification and characterisation of microorganisms within complex microbial communities. Sequence data are compared against reference databases to generate taxonomic and in some cases, functional profiles of the microbiota. The two most commonly used sequencing approaches in gut microbiome research are amplicon sequencing and shotgun metagenomic sequencing. Amplicon sequencing targets specific marker genes, such as the 16S rRNA gene in bacteria, using PCR amplification and taxon-specific primers (Moise, 2017 (Wensel et al., 2022). The 16S rRNA gene contains conserved and hypervariable regions that

enable the identification of bacterial taxa. Amplicon sequencing is widely used to assess microbial community composition and relative abundance, typically providing taxonomic identification at the genus level. However, the technique has several limitations. The choice of primers can significantly influence the accuracy of taxonomic profiling. Primers may not bind equally to all microbial taxa, which causes bias in the analysis (Klindworth et al., 2013). Another limitation is PCR amplification bias; whereby certain DNA templates are amplified more efficiently. As a result, some taxa may be overrepresented or underrepresented in the final sequencing data (Matchado et al., 2024). Among all limitations, the inability to characterise the functional potential of the microbiome is a major drawback (Jovel et al., 2016; Wensel et al., 2022). This technique characterises sequences from only a single conserved marker gene rather than the entire microbial genome. Therefore, functional profiles can only be predicted using bioinformatic tools rather than being directly measured (Wensel et al., 2022).

In contrast, shotgun metagenomic sequencing sequences all DNA present within a sample rather than targeting a specific genetic marker. Unlike 16S rRNA gene sequencing, which typically targets one or a few hypervariable regions of a single marker gene, shotgun metagenomic sequencing provides greater sequencing depth and broader genomic coverage, allowing more accurate taxonomic assignment and the direct identification of microbial functional genes (Wensel et al., 2022; Sharpton, 2014). Sequence reads can be assembled into longer contiguous sequences (contigs), improving the characterisation of individual microbial taxa and often enabling classification to the species level. Although shotgun metagenomic sequencing is more expensive than amplicon sequencing, it overcomes many of the limitations associated with amplicon-based approaches (Sharpton, 2014).

2.7 Conclusion

This literature review examines the potential effects of bioactive compounds in damson plum on the human gut microbiota. Plums contain a range of bioactive compounds, particularly

polyphenols and dietary fibre, many of which occur at higher concentrations in the skin than in the flesh. However, the composition and concentration of these compounds have not been fully characterised in New Zealand-grown damson plums. Current evidence suggests that dietary fibre and polyphenols can interact with the gut microbiota, influencing microbial composition, metabolic activity, and the production of metabolites such as SCFAs. These interactions may promote the growth of beneficial microorganisms while suppressing potentially harmful taxa. Despite growing interest in the relationship between dietary bioactive compounds and the gut microbiota, the specific effects of individual compounds remain incompletely understood. Furthermore, relatively little research has focused on damson plums (*Prunus insititia*), particularly New Zealand-grown cultivars. Overall, evidence suggests that bioactive compounds can influence gut microbial composition and function; however, the effects of bioactive compounds present in damson plums remain largely unexplored.

Chapter 3. Materials and methods

3.1 Experimental design

This study was designed and supervised by Dr Jane Mullaney (Senior scientist at Bioeconomy Science Institute, Adjunct senior research officer at Massey University) and overseen by Dr Warren McNabb (Professor of Nutritional Sciences and Leader of Sustainable Nutrition Initiative, Riddet Institute, Massey University). The laboratory work was completed by the author, Miku Sciascia, and assisted by Callum Tatton, a technical staff member at Riddet Institute.

The study employed the in vitro digestion model by Minekus et al., and the in vitro fermentation model by Edwards et al.to replicate the human gastrointestinal metabolism system (Edwards et al., 1996; Minekus et al., 2014). Four sample types were included in the study; damson plum flesh, damson plum skin-on (flesh and skin), potato starch, and pomegranate (Table 3). The sample substrates were then subjected to metagenomic sequencing to generate functional and taxonomic profiles.

Table 3. Experimental design and sample allocation for in vitro digestion and fermentation.

Sample	*n* (in-vitro digestion)	*n* (ferment)	Time	Type
Damson plum flesh	3	9	24 h	Test
Damson plum skin-on	3	9	24 h	Test
Potato starch	3	9	24 h	Control for fermentation
Pomegranate powder	3	9	24 h	Control for polyphenol

Positive control is a known polyphenol-containing fruit (pomegranate): note that we used pomegranate powder because we had it in freeze-dried form available (Zhang et al., 2023).

3.2 Samples

Damson plums were sourced from a Māori agribusiness plum producer Foot Steps Limited, Hastings, Hawke's Bay, New Zealand. They were freeze-dried and prepared in two types: skin-on and flesh-only. Potato starch was used as a control for the in vitro digestion and fermentation. The brand of freeze-dried raw organic pomegranate powder was Sunfood Superfood (U.S.A).

3.3 Nutritional analysis of the damson plum

Freeze-dried damson plum powder was sent to the Nutritional Laboratory at Massey University for evaluation of its nutritional composition.

3.4 Adult faecal collection and inoculum preparation

Massey University Human Ethics Committee approved the ethical document related to human faecal collection and fermentation in 2024 (Ethical approval number: OM1 24/27). The recruitment occurred in October 2024, and five participants signed up through the email list who had previously participated in the other in-vitro study. The questionnaires were filled out prior to sampling (Appendix A) and the consent form was filled out prior to sampling (Appendix B).

Samples were collected in the morning using the stool collection cooling box, which contained a toilet frame/sealable bucket, an anaerobic sachet, a pair of gloves, a rubbish bag, and an ice pack. The samples were delivered to the laboratory anonymously and immediately weighed and dissolved and homogenised in sterile 0.2 M Phosphate buffer (pH 6.8) to achieve 32 % (w/v). Filtered inoculum was aliquoted into tubes and frozen (-80 ^{o}C) until required.

3.5 Reagents

All reagents were purchased from Thermo Fisher and Sigma-Aldrich unless otherwise stated.

3.6 In vitro gastrointestinal digestion and fermentation

In vitro digestion and fermentation are the steps to replicate the human gastrointestinal system. For each sample, three 2.5 g aliquots were mixed with 5 mL of water in a 250 mL sample container and then subjected to simulated oral, gastric, and intestinal digestion phases. Four groups were analysed in this study. Controls: Potato starch (starch control), pomegranate (polyphenol control) and experimental samples: damson plum flesh, damson plum skin-on. Each sample was prepared in triplicate (12 samples) during the digestion stage and further run in triplicate during the fermentation stage, resulting in a total of 36 samples. A brief overview

of the process is summarized in Figure 2. The preparation of simulated digestion fluid stock solutions and complete solutions for oral, gastric and intestinal phases are presented in Table 4 and 5.

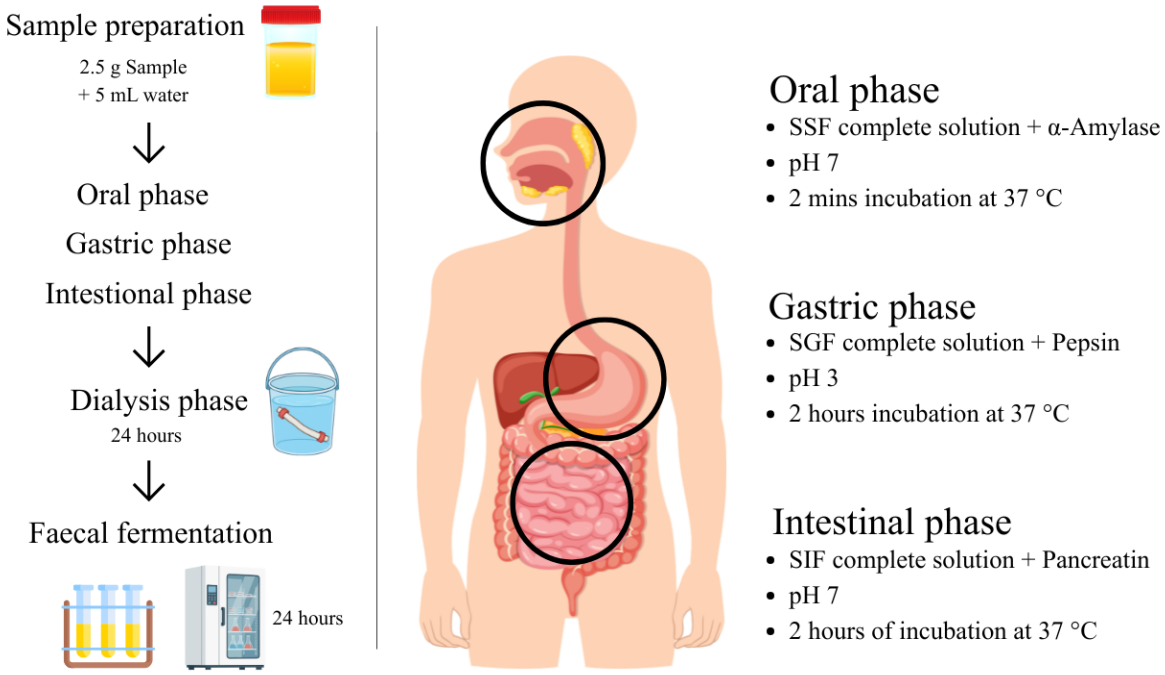


Figure 2. Brief overview of the in virto digestion and fermentation process. Created using Canva (Canva Pty Ltd., Sydney, Australia).

3.6.1 Oral phase

The following chemicals were added to the simulated salivary fluid stock solution (SSF): 2.27 mL of 0.5M KCl, 0.552 mL of 0.5 M KH2PO4, 1.020 mL of 1 M $NaHCO_3$, 0.072 mL of 0.15 M $MgCl_2(H_2O)_6$, and 0.072 mL of 0.5 M $(NH4)_2CO_3$. The solution was brought to 60.01 mL using Milli-Q water. The SSF complete solution was prepared by mixing 52.5 mL of SSF, 14.625 mL of water and 0.375 mL of $CaCl_2$. Subsequently, 4.5 mL of this prewarmed (37 °C) complete solution was added to each container containing the reconstituted sample. pH was adjusted to 7 prior to the addition of 0.5 mL α-Amylase solution. Samples were incubated for 2 minutes at room temperature. The enzyme reaction was stopped by adding 200 µl of 6M HCl, and the pH was adjusted to 3.

3.6.2 Gastric phase

The following chemicals were added to the simulated gastric fluid stock solution (SGF): 2.37 mL of 0.5M KCl, 0.311 mL of 0.5 M KH_2PO_4, 4.28 mL of 1M $NaHCO_3$. 4.05 mL of 2M NaCl, 0.112 mL of 0.15 M $MgCl_2(H_2O)_6$, and 0.174 mL of 0.5 M $(NH4)_2CO_3$. The solution was brought to 137.01 mL using Milli-Q water. The SGF complete solution was prepared mixing 10.425 mL of water, 112.5 mL of SGF, 0.075 mL of $CaCl_2$, and 24 mL of pepsin dissolved in SGF. 9.8 mL of SGF complete solution was added to each sample, followed by pH adjustment to 3. The samples were incubated for 2 hours at 37 ºC on a shaker at 150 rpm. Following 3.6.3 Intestinal phase

The following chemicals were added to the simulated intestinal fluid stock solution (SIF): 4.10 mL of 0.5 M KCl, 0.482 mL of KH_2PO_4 (0.5M), 25.61 mL of 1 M $NaHCO_3$, 5.784 mL of 2 M NaCl, and 0.663 mL of 0.15 M $MgCl_2(H_2O)_6$. The solution was brought to 241 mL using Milli-Q water. The SIF complete solution was prepared by combining 165 mL of SIF, 19.65 mL of water, 37.5 mL of Bile salts, 0.6 mL of $CaCl_2$, and 75 mL of Pancreatic solution made in SIF. 19.85 mL was added to each sample, and the pH was adjusted to 7. The incubation time was 2 hours for this stage, held at 37 °C, on a shaking incubator at 150 rpm. The samples were boiled at 95 °C for 5 mins to neutralize enzyme reaction.

After the intestinal phase, samples underwent dialysis to separate compounds according to component sizes and simulate nutrient absorption using dialysis tubing. The samples were then fermented in a faecal slurry for 24 hours to replicate colon fermentation. Samples were collected at 0 hours (pre-fermentation) and 24 hours (post-fermentation) to compare microbial composition. Thereafter, fermented samples were analysed using the downstream processing method.

Table 4. Preparation of simulated digestion fluid stock solutions.
Adapted from (Minekus et al., 2014).

		SSF (15 digests)		SGF (15 digests)		SIF (15 digests)	
Constituent	Stock Conc. (mol L-1)	Vol. of stock	Conc. in SSF (mmol L^{-1})	Vol. of stock	Conc. in SGF (mmol L^{-1})	Vol. of stock	Conc. in SIF (mmol L^{-1})
KCl	0.5	2.27	15.1	2.37	6.9	4.1	6.8
KH_2PO_4	0.5	0.552	3.7	0.311	0.9	0.482	0.8
$NaHCO_3$	1	1.02	13.6	4.28	25	25.61	85
NaCl	2	-	-	4.05	47.2	5.784	38.4
$MgCl_2(H_2O)_6$	0.15	0.072	0.15	0.112	0.5	0.663	0.33
$(NH4)_2CO_3$	0.5	0.072	0.06	0.174	0.5	-	-
Total		**4**		**11.3**		**36.63**	
Add H_2O (MilliQ)		56.02		125.72		204.37	
Final Volume		**60.01**		**137.01**		**241**	

Note: SSF (Simulated Salivary Fluid), SGF (Simulated Gastric Fluid), and SIF (Simulated Intestinal Fluid)

Table 5. Preparation of complete simulated digested fluid solution.
Adapted from (Minekus et al., 2014).

SSF complete	(mL)	SGF complete	(mL)	SIF complete	(mL)
SSF solution	52.5	H2O	10.425	SIF	165
H2O	14.625	SGF	112.5	H2O	19.65
CaCl2	0.375	CaCl2	0.075	Bile salts	37.5
Total volume	67.5	Pepsin in SGF	24	CaCl2	0.6
		Total volume	147	Pancreatic solution made in SIF	75
				Total volume	297.75

3.7 DNA extraction and metagenomic analysis

DNA was extracted from pre- and post-fermentation samples using a NucleoSpin Soil kit (Item number: 740780.50) from Macherey-Nagel (Düren, Germany). DNA concentration was measured on a NanoDrop spectrophotometer. The selected 27 samples (3 pre-fermentation and 24 post-fermentation) were sent for metagenomic sequencing.

3.8 SCFAs production analysis

3.8.1 SCFAs extraction

SCFAs were extracted using a liquid-liquid extraction method. The samples were first centrifuged to separate solid and liquid materials. 100 µl of 50 mM 2-ethyl butyric acid solution (internal standard) was added to 900 µl of fermentation supernatant. From this mixture, 500 µl was transferred into a fresh tube, and 1250 µl of Diethyl Ether and 250 µl of concentrated HCl (37 %) were added. This solution was mixed vigorously prior to fatty acid derivatisation. 200 µL of the extract was transferred to a GC class vial, and 40 µL of MTBSTFA (N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide) was added. After attaching a cap to every vial, the extracts were incubated at 80 ºC for 20 minutes. Samples were cooled on ice then left at room temperature for up to 72H to ensure complete derivatization.

3.8.2 Gas chromatography

The extracted SCFAs were analysed on Nexus GC-2010 Gas Chromatography Flame Ionisation Detection (GC-FID) system with a HP-1 column (30m x 0.25mm ID,0.25 µm film thickness). The column was purchased from Agilent Technologies, USA. Helium was used as the carrier gas operated at 131.2 kPa, with a flow rate of 52.3 mL/min and a split ratio of 30:1. The detector temperature was set to 310°C. The oven was initially set at 70°C and heated to 115°C at 6°C/min, followed by a rapid increase to 280°C at 60°C/min. After a 3 min hold at 280°C, the temperature was reduced to 70°C at 120°C/min. The data was then analysed through Shimadzu LabSolutions software (v5.98). The data were exported to Microsoft Excel

spreadsheets for further analysis after integrating peak. The peak area of each SCFA was normalised to the internal standard, and calibration curves were created using external standards. Concentrations of individual SCFAs were determined from the linear regression equations obtained from the calibration curves.

3.9 Statistical analysis

3.9.1 SCFA statistical analysis

The SCFAs analysis was conducted by Dr Jane Mullaney (Bioeconomy Science Institute – AgResearch) using Graphpad Prisms version 10.6. Differences in SCFA concentrations among treatment groups were assessed using the non-parametric Kruskal-Wallis test, followed by Dunn's test to compare the mean rank of each treatment groups against the mean rank of the control.

3.9.2 Microbiome analysis and statistics

Shotgun metagenomic sequencing was conducted by Annoroad Gene Technology Co., Lid. (Beijing, China) on an Illumina NovaSeq X Plus (Illumina, Inc., San Diego, CA, USA). Raw sequencing data were processed by Dr Jane Mullaney (Bioeconomy Science Institute – AgResearch) using R version 4.4.0 (2024-04-24 ucrt) and using the bioBakery suite of tools (McIver et al., 2018). Taxonomic profiling was performed using MetaPhlAn 4.2.4 whereas functional potential profiling was performed using HUMAnN 3.0, with UniRef90 gene families (clusters of protein sequences with ≥90% sequence identity (Suzek et al., 2015), and converted to KEGG ortholog groups (Kanehisa et al., 2017). Data were normalised to counts per million (CPM).

Multivariable association analysis

Associations between microbial features (taxonomy or functional potential) and their interaction (food type) were examined using multivariable linear mixed models fitted with Microbiome Multivariable Associations with Linear Models 3 (MaAsLin3 version 3.0.0)

(Nickols et al., 2024). Substrate type was included as a fixed effect, with potato starch used as the reference level, and replicate was included as a random effect. Models were fitted using the abundance framework with no additional normalisation, log-transformed feature abundances and a minimum prevalence threshold of 10. Plots were made with R package ggplot2 version 4.0.2.

Taxonomic composition analysis

Statistical analyses were performed R version 4.4.0 (2024-04-24 ucrt). Multiple testing correction was performed using the Benjamini–Hochberg false discovery rate method (significance set at $q < 0.1$). Microbiome data were analysed with R package phyloseq version 1.48.0. Alpha (Shannon, Simpson) and beta diversity (based on Bray-Curtis distance matrices) indices were calculated from MetaphlAn species-level tables and the R package vegan version 2.6-8. Changes in alpha diversity indices within groups after the study intervention were analysed using paired Wilcoxon signed-rank tests and differences in changes in alpha diversity indices between groups were analysed using linear mixed models. PrinciPCoA were plotted using Bray-Curtis and Aitchison distance matrices. Differences in changes in community composition and functional potential over time between groups were determined by performing PERMANOVA on Bray-Curtis and Aitchison distance matrices using adonis2 from vegan. Variability in group dispersion was assessed using betadisper from vegan. Figures and tables generated were then interpreted and analysed by the author.

Chapter 4. Results

4.1 Damson plum nutritional value

The nutritional values of damson skin-on and flesh are summarised in Table 6. Damson skin-on had higher crude protein and TDF whereas damson flesh had higher ash, fat, starch, and carbohydrate contents.

Table 6. Damson plum nutritional value (g/100g dry matter).

Sample type	Ash	Crude Protein	Fat	Starch	Carb by Calc	TDF
Damson skin-on	3.523	3.316	5.596	0.207	77.617	9.845
Damson flesh	6.250	2.917	5.625	0.313	78.646	6.563

Note: TDF (Total dietary fibre)

4.2 Taxonomic composition

4.2.1 Alpha diversity

Alpha diversity varied among substrate treatments (Figure 3). Both Shannon and Simpson diversity indices were higher in the damson flesh and damson skin-on treatments than in the potato starch treatment, indicating greater microbial diversity and evenness in communities exposed to damson substrates. Pomegranate treatments exhibited intermediate diversity values and greater variability among replicates. Overall, damson treatments were associated with higher alpha diversity than the potato starch control.

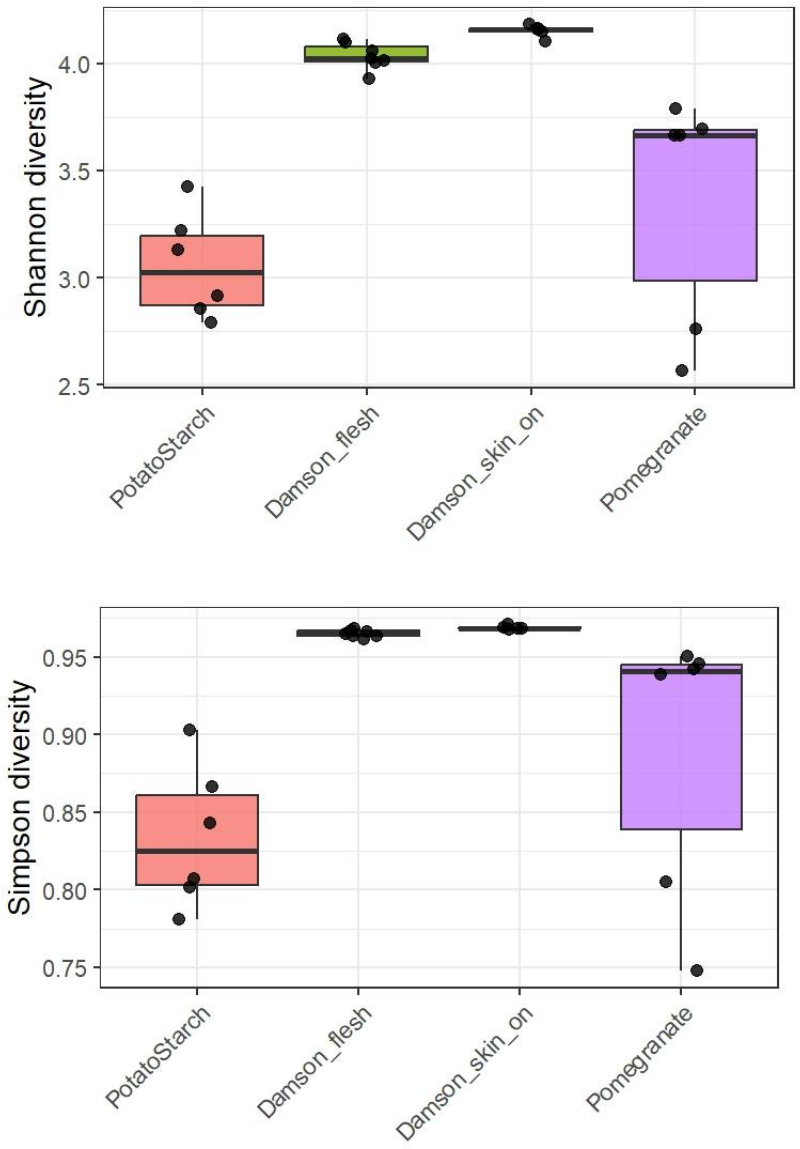


Figure 3. Alpha diversity of microbial communities across substrate treatments following 24 h incubation.
Shannon diversity (top) and Simpson diversity (bottom) indices were calculated from MetaPhlAn species-level profiles. Boxplots show the median and interquartile range, with individual points representing biological replicates. Treatments included potato starch, damson flesh, damson skin-on, and pomegranate substrates.

Post hoc Dunn's tests identified significant differences in alpha diversity among substrate treatments (Table 7). Both Shannon and Simpson diversity indices were significantly higher in damson flesh and damson skin-on treatments than in the potato starch control. Damson skin-on treatments also exhibited significantly higher diversity than pomegranate treatments for both indices, whereas damson flesh differed significantly from pomegranate only for Shannon

diversity. No significant differences were observed between damson flesh and damson skin-on treatments, and pomegranate did not differ significantly from potato starch for either diversity metric. Overall, the damson substrates were associated with higher microbial alpha diversity than the potato starch control.

Table 7. Benjamini–Hochberg adjusted p-values from Dunn's post hoc pairwise comparisons of Shannon and Simpson alpha diversity indices across substrate treatments.
Significant differences (adjusted $p < 0.05$) indicate differences in microbial alpha diversity between treatment groups following 24 h incubation.

Comparison	Shannon	Simpson
PotatoStarch vs Damson flesh	0.014	0.011
PotatoStarch vs Damson skin-on	0.001	0.001
PotatoStarch vs Pomegranate	0.624	0.514
Damson flesh vs Damson skin-on	0.206	0.206
Damson flesh vs Pomegranate	0.042	0.052
Damson skin-on vs Pomegranate	0.003	0.003

4.2.2 Beta diversity

Beta diversity analysis based on Bray–Curtis dissimilarities revealed significant differences in microbial community composition among treatments (PERMANOVA, $F_{3,20} = 20.79$, $R^2 = 0.757$, $P = 0.001$). A test for homogeneity of multivariate dispersion indicated no significant differences in within-group dispersion among treatments (PERMDISP, $F_{3,20} = 2.27$, $P = 0.099$), suggesting that the PERMANOVA result reflected differences in community composition rather than differences in group variability. Pairwise PERMANOVA comparisons identified significant differences between several treatment pairs (Table 8).

Table 8. Pairwise PERMANOVA results for microbial community composition based on Bray–Curtis dissimilarities across substrate treatments.
P-values were adjusted for multiple testing using the Benjamini–Hochberg (BH) false discovery rate correction. R^2 indicates the amount of variation explained by the comparison, F is the test statistic, and P is the p-value.

Comparison	R^2	F	P	BH-adjusted p
Damson skin-on vs Damson flesh	0.385	6.26	0.006	0.0072
Pomegranate vs Damson flesh	0.502	10.08	0.002	0.0060
Pomegranate vs Damson skin-on	0.561	12.8	0.004	0.0060
Potato starch vs Pomegranate	0.424	7.36	0.02	0.0200
Potato starch vs Damson flesh	0.893	83.32	0.001	0.0060
Potato starch vs Damson skin-on	0.894	84.04	0.004	0.0060

Potato starch and pomegranate samples clustered separately from the damson treatments along the first principal coordinate axis, indicating compositional differences between damson and the other groups. In contrast, the potato starch and pomegranate showed substantial overlap (Figure 4). Damson flesh and damson skin-on samples clustered closely together, whereas pomegranate samples exhibited greater dispersion and variability among replicates. These results indicate that substrate type was an important determinant of microbial community composition.

Genus-level community composition varied among substrate treatments following 24H incubation (Figure 5). Three 0H samples (Po3a0H, Dp1c0H and Pg1c0H) exhibited similar microbial compositional patters. The 24H damson flesh and damson skin-on treatments exhibited broadly similar community profiles, characterised by lower relative abundances of *Bifidobacterium* and higher relative abundances of genera including *Faecalibacterium*, *Enterococcus*, *Mediterraneibacter* and *Bacteroides* compared with the potato starch treatment. Potato starch samples generally contained higher proportions of *Bifidobacterium*, although substantial variation was observed among individual samples. Pomegranate samples displayed greater between-sample variability than the other treatments, with some samples exhibiting community profiles similar to potato starch and others more closely resembling the damson

treatments. Overall, differences among substrate treatments were more apparent than differences associated with the presence or absence of damson skin.

Differential abundance analysis (MaAsLin3; $q<0.1$) identified a relatively small number of genera that differed between damson skin-on and damson flesh treatments, including higher abundances of *Lachnospira*, *Vescimonas*, and *Escherichia* in the skin-on treatment. In contrast, comparisons between damson and pomegranate treatments identified a larger number of differentially abundant genera. Pomegranate treatments were associated with higher abundances of *Bifidobacterium* and *Lactobacillus*, whereas damson treatments showed higher abundances of genera including *Lachnospira, Dorea*, and *Vescimonas*. Complete differential abundance results for the skin-on versus flesh and skin-on versus pomegranate comparisons are shown in Appendix A.

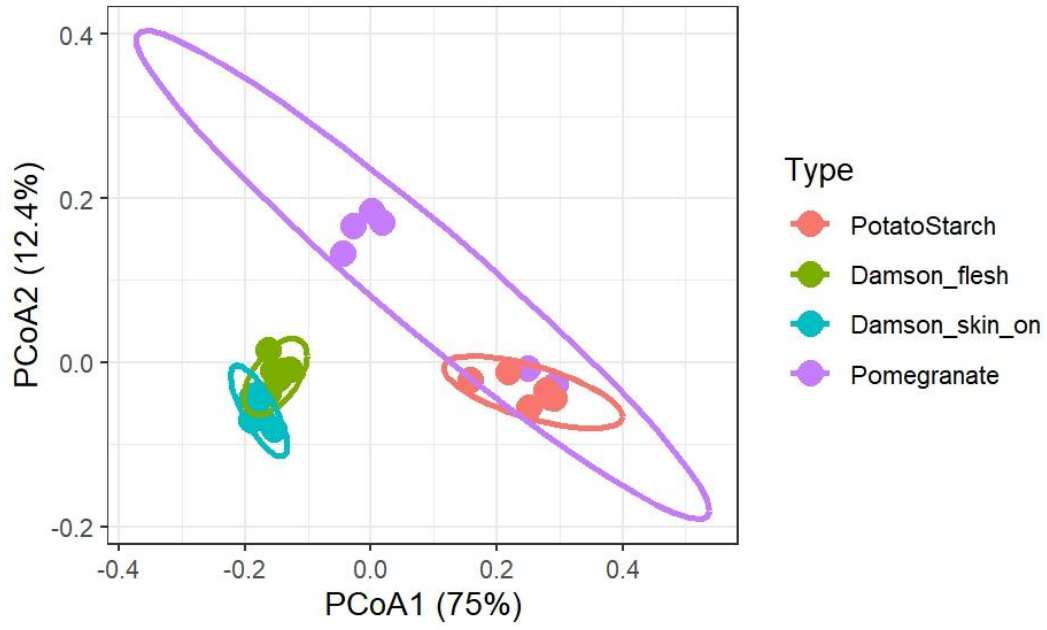


Figure 4. Principal coordinates analysis (PCoA) of Bray–Curtis dissimilarities calculated from MetaPhlAn species-level profiles following 24 h incubation.
PC1 and PC2 explained 74.8% and 12.5% of the variation, respectively. Damson flesh and damson skin-on samples clustered closely together, whereas potato starch and pomegranate formed distinct clusters. Greater dispersion was observed among pomegranate replicates compared with the other treatment groups.

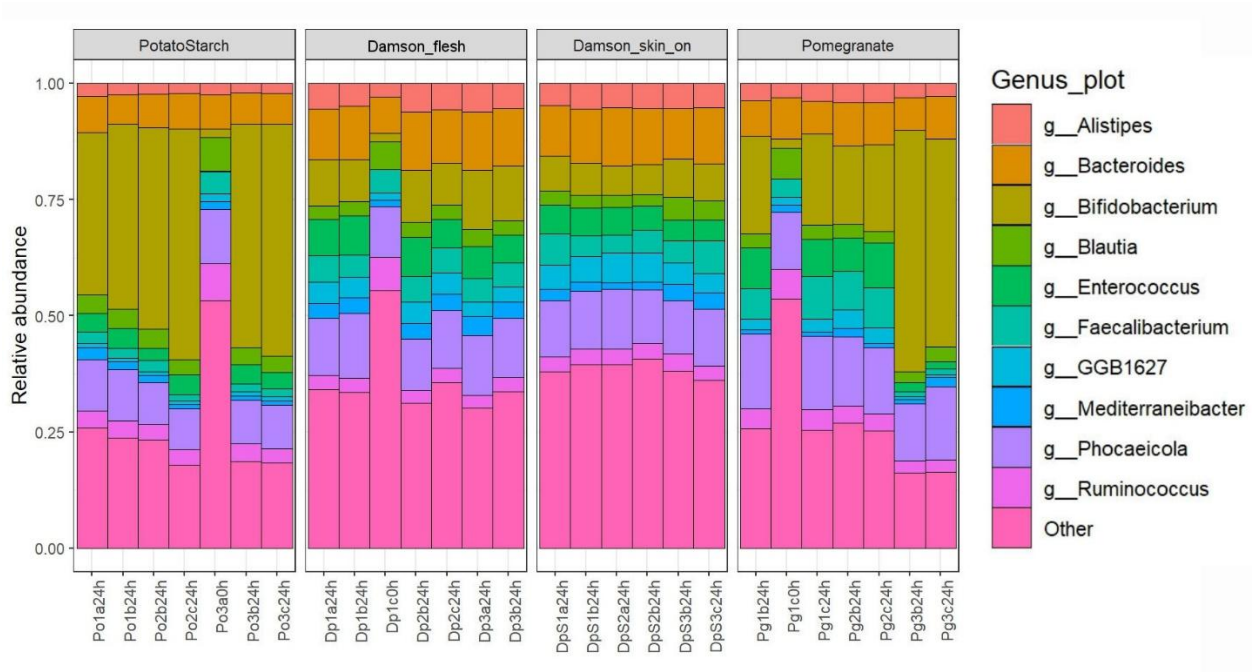


Figure 5. Relative abundance of the ten most abundant bacterial genera across substrate treatments following 24 h incubation.
Bars represent individual samples and are coloured by genus-level taxonomic assignment. Taxa outside the ten most abundant genera were grouped as "Other". Treatments included potato starch, damson flesh, damson skin-on, and pomegranate substrates. Relative abundances are shown as proportions of the total bacterial community within each sample. Three Time 0Hs are shown (Sample IDs: Po3a0H, Dp1c0H and Pg1c0H) in potato starch, damson flesh, damson skin-on and in pomegranate as a reference to the starting inoculate.

Relative enrichment analysis highlighted substrate-specific taxonomic patterns (Figure 6). Damson flesh and damson skin-on exhibited different enrichment profiles, consistent with the limited number of genera identified as differentially abundant between these treatments. In contrast, potato starch showed relative depletion of several genera enriched in damson and pomegranate substrates, including *Veillonella*, *Parasutterella*, *Lawsonibacter*, and *Anaerobutyricum*. However, *Bifidobacterium* was more strongly enriched in the potato starch and pomegranate treatments. Pomegranate displayed a moderate enrichment profile for many genera.

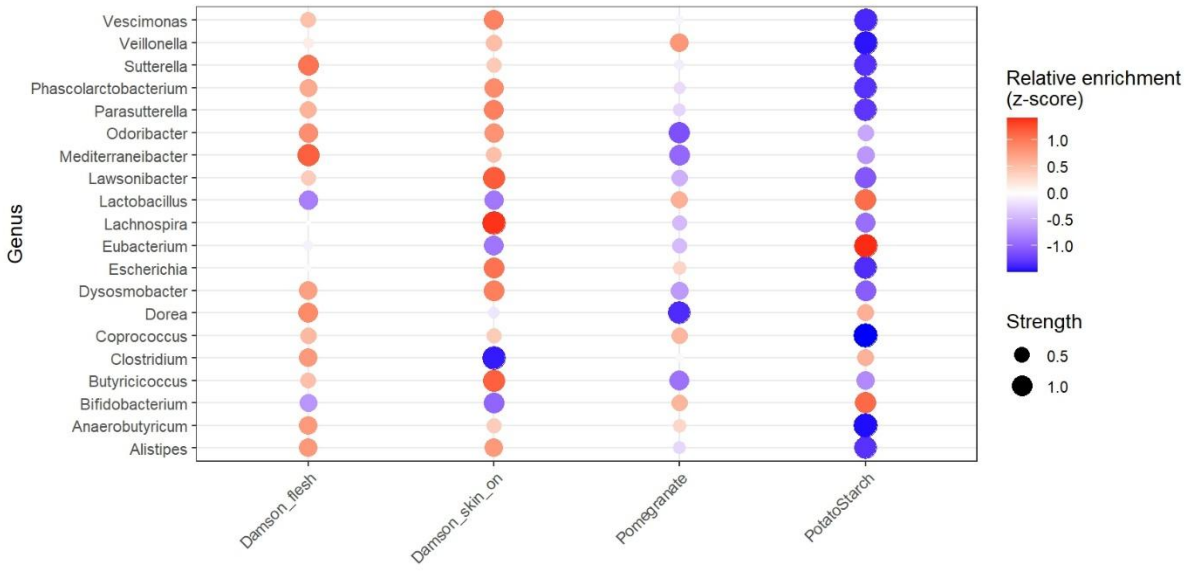


Figure 6. Bubble plot showing relative enrichment (red) or depletion (blue) of selected bacterial genera across the experimental substrates.
Values represent z-score transformed log10 mean relative abundances, scaled within each genus to show substrate-specific enrichment patterns relative to each taxon's own average abundance. Circle size indicates the magnitude of enrichment or depletion. Damson flesh and damson skin-on generally showed similar enrichment patterns, whereas potato starch was associated with depletion of several genera including Veillonella, Parasutterella, Lawsonibacter, and Anaerobutyricum. In contrast, Bifidobacterium and Eubacterium showed relative enrichment in potato starch and pomegranate treatments. Genera shown were identified as significantly differentially abundant in MaAsLin3 analyses (q < 0.1). Mean relative abundances were log10 transformed and z-score scaled within each genus to visualise relative enrichment patterns across substrates.

4.3 Functional analysis

Correlation analysis revealed a moderate positive relationship between KEGG ortholog $\log_2$ fold changes in the damson skin-on versus flesh comparison and the damson versus pomegranate comparison (r = 0.60; Figure 7). Most orthologs had negative log2 fold changes in both comparisons, indicating lower representation in skin-on relative to flesh and in damson relative to pomegranate. However, the larger spread was observed in damson relative to pomegranate, highlighting that changes were larger for damson vs pomegranate than skin-on vs flesh. This suggests that substrate type had a stronger influence on predicted functional profiles than the presence or absence of damson skin.

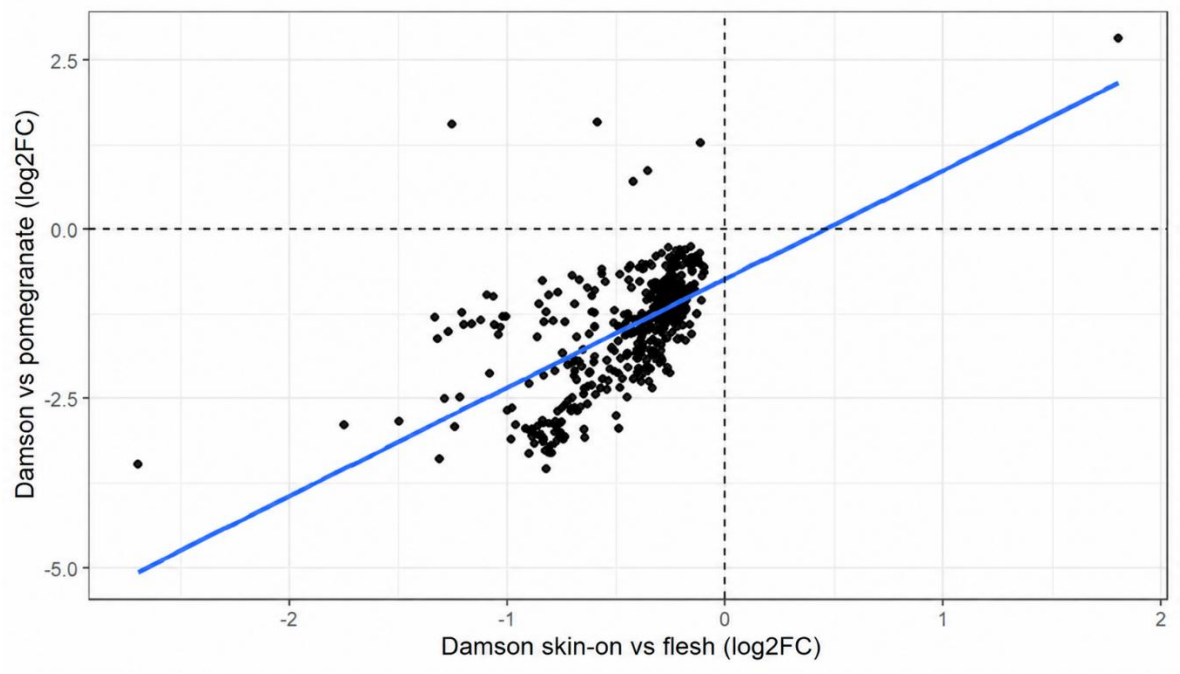


Figure 7. Concordance of functional responses across substrate comparisons.
Scatter plot showing KEGG ortholog log_2 fold changes across substrate comparisons. The x-axis represents log_2 fold changes for damson skin-on relative to damson flesh treatments, whereas the y-axis represents log_2 fold changes for damson relative to pomegranate treatments. Each point represents a KEGG ortholog. Dashed lines indicate no change ($log_2FC = 0$). The blue line shows the fitted linear regression (Pearson correlation, $r = 0.60$).

Functional profiling revealed distinct differences in microbial metabolic potential across substrates. As shown in Figure 8, comparison of damson treatments with pomegranate demonstrated a broad reduction in KEGG ortholog abundance across multiple functional categories. Pathway-level summarisation showed that the majority of KEGG orthologs within central metabolic pathways—including carbohydrate metabolism, membrane transport, and stress response—were decreased in damson, with 86–100% of orthologs exhibiting negative log_2 fold changes. Despite this general reduction, the magnitude of change differed between comparisons. A moderate correlation (r = 0.60) between damson skin vs flesh and damson vs pomegranate contrasts indicated that while directional trends were partially conserved, the dominant source of variation was substrate type rather than the presence of skin. Comparison of damson flesh with potato starch revealed a contrasting pattern (Figure 9). While most central

metabolic pathways were reduced in damson flesh, aromatic/phenolic metabolism was enriched, representing the only pathway with a positive median $\log_2$ fold change. This indicates a shift towards specialised metabolic functions associated with aromatic compound processing. Together, these results indicate a shift in functional profile away from central metabolic pathways and towards aromatic/phenolic metabolism. To assess whether these differences were driven by low-abundance features, KEGG ortholog abundance and prevalence were examined across groups. Significant KEGGs were consistently detected across samples, with the majority present in ≥66% of samples per group and several detected in all samples. In addition, these features exhibited moderate to high relative abundance rather than near-zero values. Together, this indicates that the observed functional differences were not driven by sparse or low-abundance artefacts but reflect consistent and biologically meaningful shifts in microbial functional profiles.

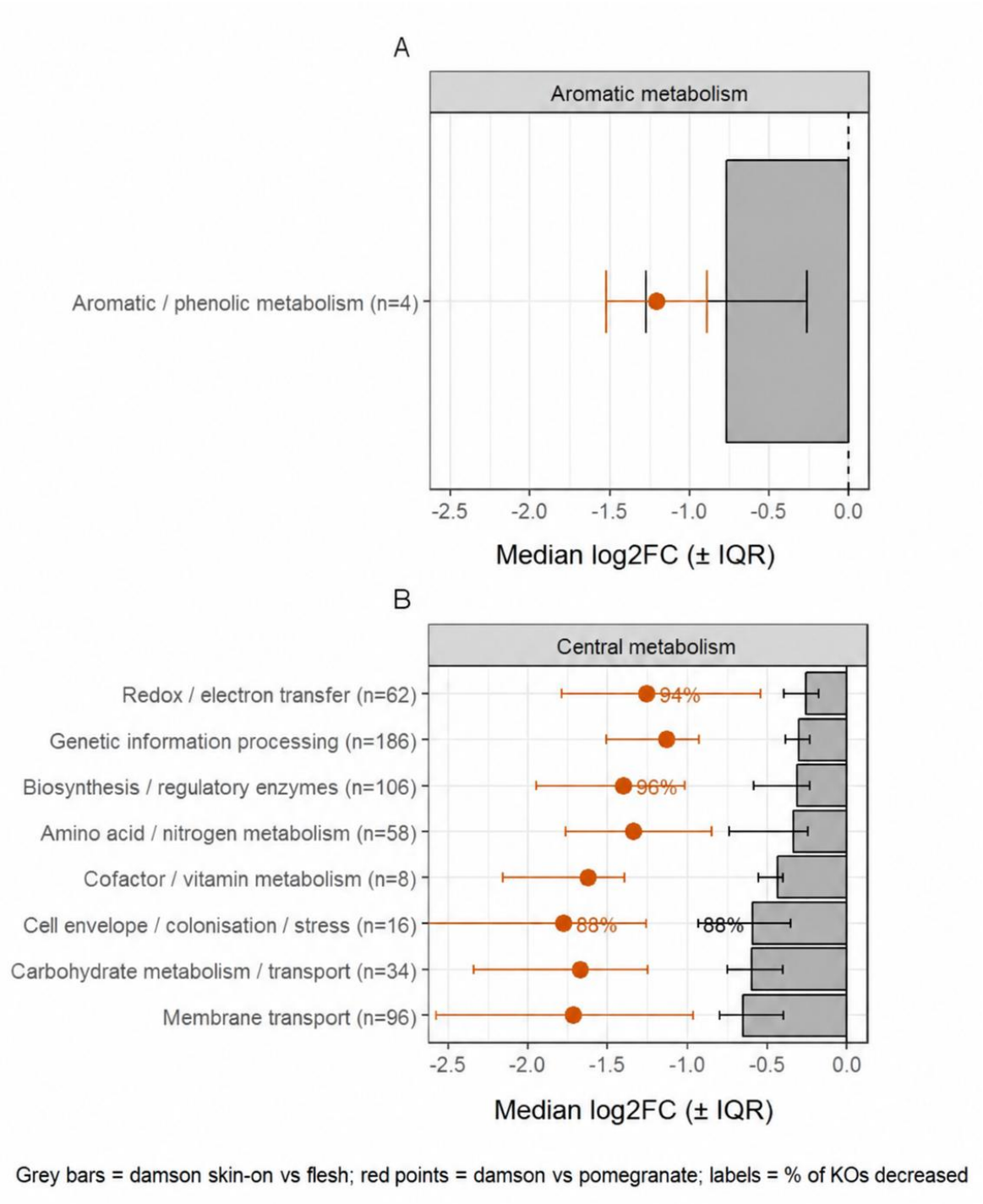


Figure 8. Pathway-level shifts in damson relative to pomegranate.
(A)Aromatic and phenolic metabolism responses comparing damson (flesh or skin-on) to pomegranate. (B)Summary of central metabolism pathway responses comparing damson to pomegranate. Grey bars represent the median log_2 fold change (± IQR) for damson skin-on relative to damson flesh, and orange points represent combined damson treatments relative to pomegranate. Labels indicate the percentage of KEGG orthologs within each pathway that are decreased. Across most pathway categories, median KEGG ortholog abundance was lower in damson than pomegranate treatments, with 86–100% of orthologs showing negative log_2 fold changes within central metabolic categories.

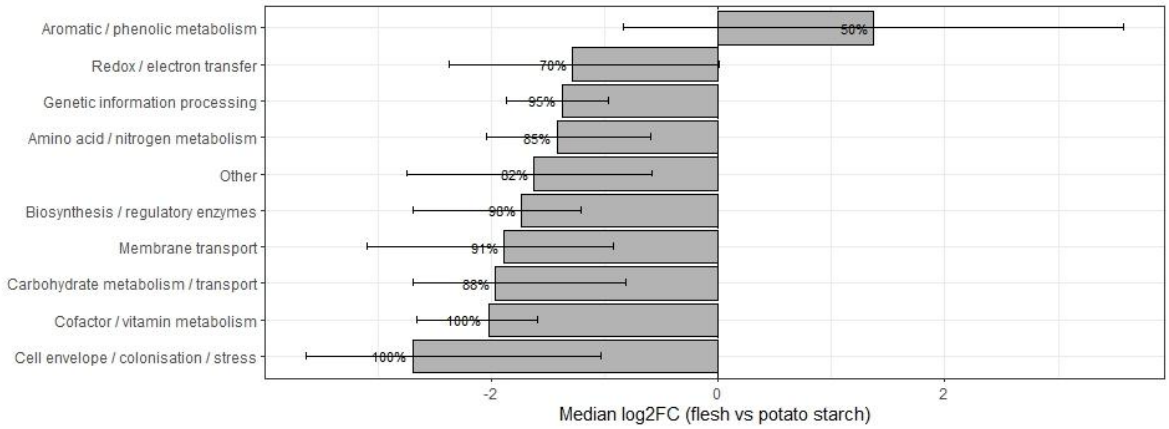


Figure 9. Functional response of damson flesh relative to potato starch.
Median log_2 fold changes (± IQR) of KEGG pathways comparing damson flesh to potato starch. Negative values indicate reduced functional abundance in damson flesh. Most pathways show decreased abundance, including carbohydrate metabolism, membrane transport, and stress-related functions. In contrast, aromatic/phenolic metabolism was the only pathway category with a positive median log_2 fold change, indicating greater representation of KEGG orthologs associated with aromatic compound processing.

4.4 Short-chain fatty acids

Potato starch generated the highest acetate concentrations, likely reflecting fermentation of its starch fraction. In contrast, propionate, butyrate, lactate, and succinate concentrations were comparatively low. Damson skin-on and flesh produced lower acetate concentrations than potato starch but higher concentrations of both propionate and butyrate. However, these treatments produced little to no lactate or succinate. Pomegranate showed a similar fermentation profile to the damson treatments, with relatively lower acetate production and higher butyrate concentrations. The concentrations of the other SCFAs in pomegranate were intermediate to those observed for potato starch and damson. The greatest variation was observed in the pomegranate treatment. (Figure 10).

PCoA of SCFA profiles as shown in Figure 11 revealed separation among treatment groups. Pomegranate treatment was more widely dispersed than the other treatment groups, indicating greater variability. In contrast, damson skin-on and flesh clustered closely together, suggesting

similar SCFA profiles between treatments. Potato starch samples were separated from the damson treatment, demonstrating differences in SCFA compositions.

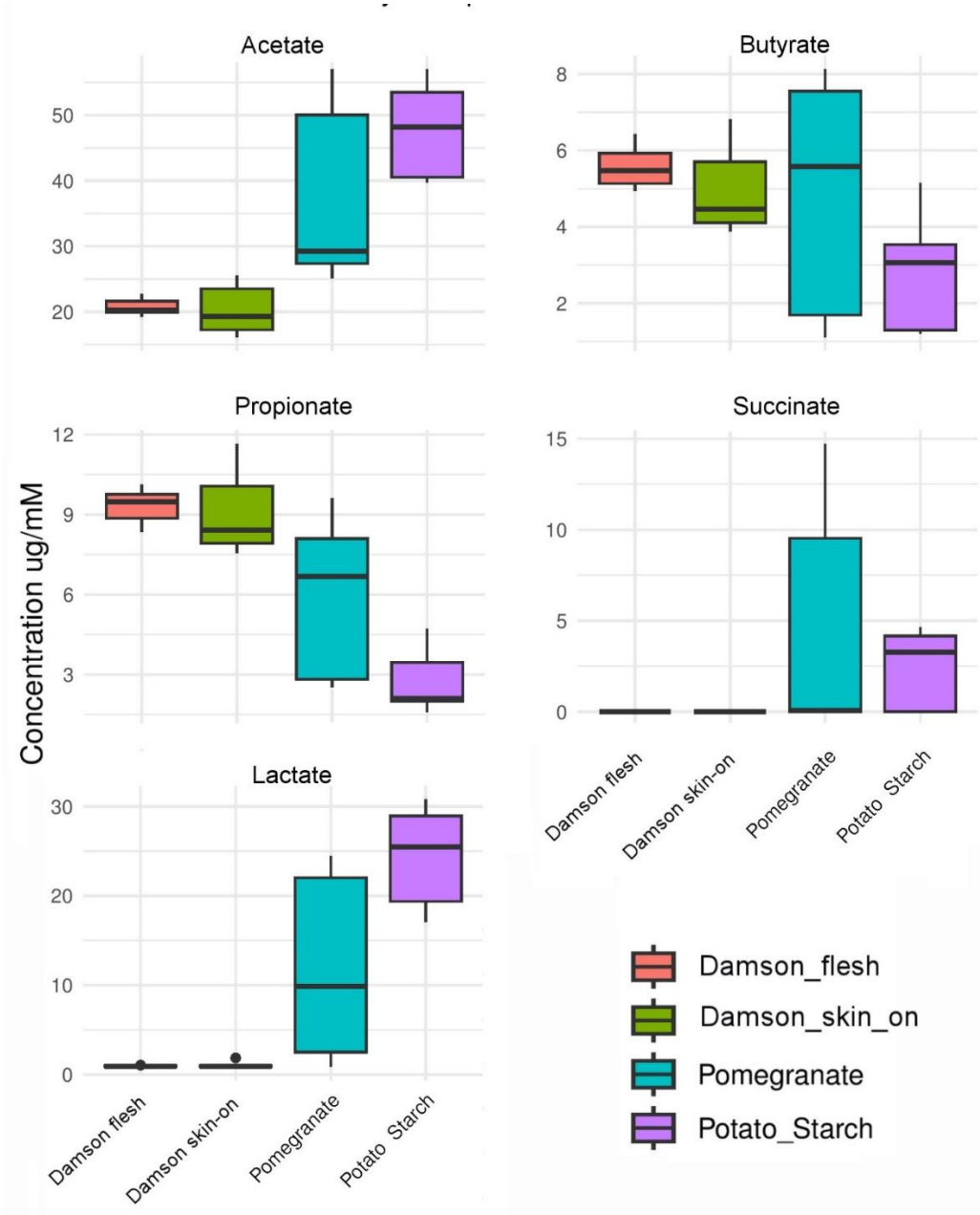


Figure 10. Concentrations of SCFAs among treatment groups.
Concentrations of acetate, butyrate, lactate, propionate, and succinate measured following in vitro faecal fermentation of damson flesh, damson skin-on, pomegranate, and potato starch substrates. Data are presented as boxplots, where the centre line indicates the median, boxes represent the interquartile range (IQR), whiskers extend to 1.5 × IQR, and points represent outliers.

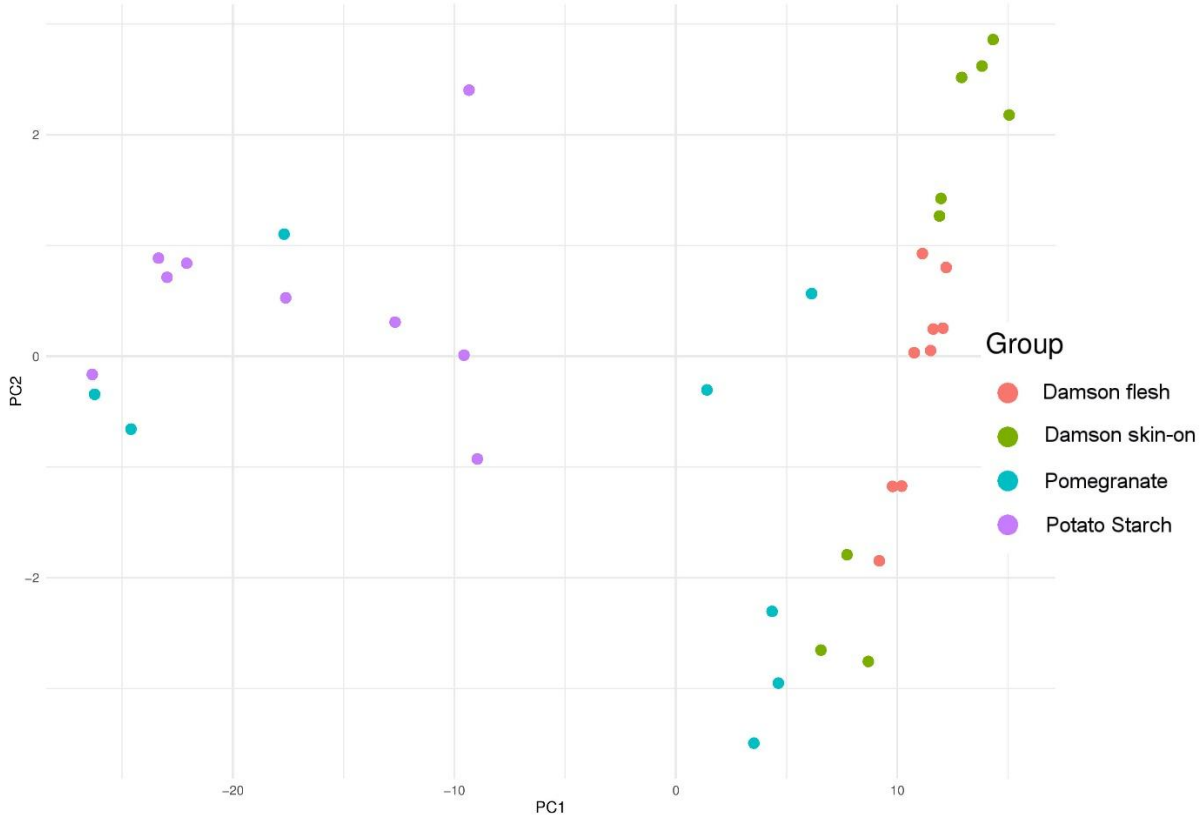


Figure 11. PCoA of SCFA profiles following 24 h in vitro fermentation.
Each data points represent individual samples (n = 9 per treatment group) and colour represent each group, damson flesh, damson skin-on, pomegrante, and potato starch. Samples that are clustering closer together exhbit more similar SCFA profiles, while greater distacne indicates differences in SCFA composition between points.

Significant differences in SCFA production were observed among the substrate treatments (Figure 12). Acetate concentrations were significantly higher in 24H fermentation of potato starch compared with both damson treatments ($p < 0.001$) and the pomegranate treatment ($p < 0.05$). In contrast, butyrate and propionate concentrations were significantly higher in both damson treatments than in potato starch fermentations ($p < 0.01$ and $p < 0.001$, respectively). Pomegranate produced a broadly similar SCFA profile to the damson treatments; however, greater variability was observed among replicates for butyrate and propionate concentrations.

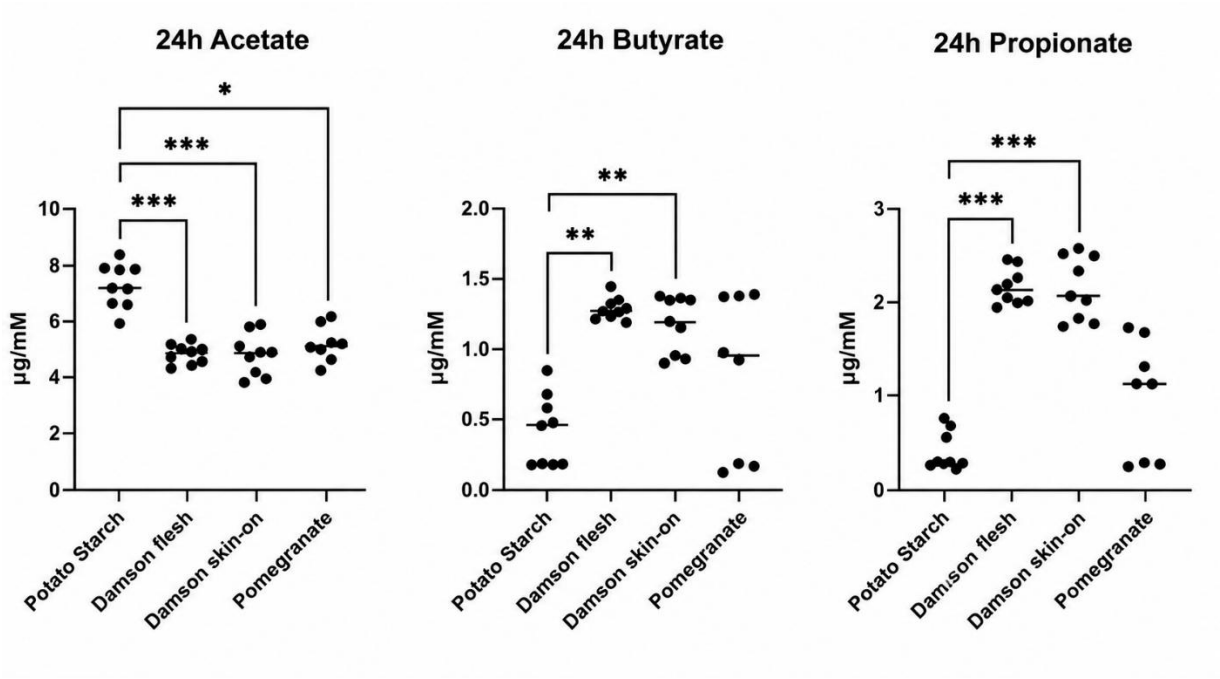


Figure 12. Production of three major SCFAs, acetate, butyrate, and propionate, following 24h incubation.
Statistical significance is indicated as follows: p-value <0.05 (*), p-value <0.01 (**) and p-value <0.001 (***).

Chapter 5. Discussion and conclusion

5.1 Discussion

Little is known about the health benefits of consuming damson plums. A Scopus search (June 2026) using the term "damson plums" retrieved only eight publications, including a book chapter on plums, with just one study investigating the bioactive composition of *Prunus* species (Popović et al., 2021). In that study, plum cultivars were compared for their bioactive composition and antioxidant properties. Although damsons were not identified as having the highest overall bioactive potential, they contained the highest concentrations of total phenolics and flavonols among the cultivars examined (Popović et al., 2021). Total phenolics were estimated using the Folin–Ciocalteu (FC) assay, while antioxidant capacity was assessed using the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. While these assays are useful for comparing the chemical antioxidant properties of foods, they do not measure biological activity and cannot be used to predict health effects in humans.

This research investigated the effect of bioactive compounds in New Zealand-grown damson plums on the human gut microbiome. damson plum treatments produced distinct microbial communities in this in vitro fermentation model. Our results have demonstrated that damson plums were associated with selective enrichment of aromatic and phenolic metabolism despite widespread reductions across central metabolism functions.

Taxonomic analysis revealed that substrate types influenced microbial community composition, whereas the presence of damson skin had a relatively limited effect relative to damson flesh. Damson flesh and skin-on treatments exhibited highly similar microbial profiles, suggesting the microbial response was driven primarily by the damson substrate type rather than a presence or absence of skin. Pomegranate had a greater variability among replicates; however, the composition differed from other substrate types.

Changes in microbial community composition may influence microbial metabolic activity and the production of fermentation-derived metabolites. Therefore, SCFA production was examined to evaluate the metabolic responses of the microbial communities to the different substrates. High acetate production under potato starch treatment was consistent with previous studies demonstrating increased microbial fermentation of resistant starch substrates (Lesmes et al., 2008; Muir et al., 1995). The butyrate and propionate profiles observed in pomegranate and damson suggested comparable effects of polyphenol-rich components on microbial fermentation activity. In addition, damson flesh and skin-on exhibited similar acetate, butyrate, and propionate production, indicating that the presence of skin did not substantially alter acetate, butyrate, or propionate production relative to flesh alone. Pomegranate is particularly rich in ellagitannins, high-molecular-weight polyphenols found in fruits, nuts, and seeds (e.g., pomegranates, berries, walnuts) that act as natural plant defences. As hydrolysable tannins, they release ellagic acid, which gut microbiota convert into health-promoting urolithins, offering potential antioxidant, anti-inflammatory, and anti-cancer benefits (Banc et al., 2023).

Microbial functional profiling revealed that substrate composition influenced community metabolic potential, with a clear separation between pomegranate, damson-derived substrates, and potato starch. Across comparisons, damson treatments were associated with a broad reduction in KEGG ortholog abundance relative to pomegranate, affecting multiple central metabolic pathways, including carbohydrate metabolism, membrane transport, redox processes, and stress response. Despite the reduction, damson substrates showed greater representation of KEGG orthologs associated with aromatic and phenolic compound metabolism compared with potato starch. This finding indicates that functional differences among substrates were pathway-specific rather than affecting all functional categories to the same extent. This observation is consistent with the higher polyphenol content of plums relative to starch-based

substrates and the known involvement of gut microbial communities in the transformation of dietary phenolic compounds (Rodríguez-Daza et al., 2020; Wang et al., 2022).

Polyphenols are well recognised for their antimicrobial and growth-modulating properties, often inhibiting broad microbial activity while favouring taxa and functions adapted to their degradation (Neyrinck et al., 2013; Parkar et al., 2008). The enrichment of aromatic metabolism observed here is consistent with this mechanism, suggesting that damson-derived compounds impose selective pressure on the microbial community. Under these conditions, microbes capable of metabolising aromatic substrates may gain a relative advantage, even as the representation of genes associated with central metabolic pathways was reduced.

Importantly, the observed functional shifts were supported by consistent detection across samples and moderate relative abundance, suggesting that these patterns were not driven by low-abundance random variations. The positive correlation between the skin-on versus flesh and damson versus pomegranate comparisons suggests that many KEGG orthologs changed in the same direction across both comparisons. However, the substantially larger effect sizes observed in the damson versus pomegranate comparison suggest that substrate type exerted a stronger influence on microbial functional profiles than skin inclusion.

Taken together, these findings suggest that damson-derived substrates were associated with reduced representation of KEGG orthologs across multiple metabolic categories while exhibiting enrichment of functions associated with aromatic compound processing. In contrast, pomegranate was associated with greater representation of KEGG orthologs across a broader range of functional categories, whereas potato starch maintained a more generalised functional profile without selective enrichment of aromatic compound-associated functions. These findings highlight the importance of substrate composition in shaping microbial functional profiles and suggest that polyphenol-rich substrates may selectively influence specific functional categories rather than affecting all pathway categories to the same extent.

5.2 Conclusion

This study investigated whether damson plum skin hypothesised to contain higher concentrations of bioactive compounds than damson plum flesh, differentially influenced gut microbial composition and functional profiles during *in vitro* fermentation. The results demonstrated that substrate type was associated with distinct microbial community and functional responses supported by differences in SCFA production, alpha diversity, beta diversity, taxonomic composition, and KEGG functional profiles. Functional profiling indicated broad reductions in the representation of central metabolic pathways in damson treatments relative to pomegranate and potato starch, alongside enrichment of aromatic and phenolic metabolism pathways. Similar responses were observed between damson flesh and skin-on treatments, suggesting that the presence of skin did not substantially alter microbial community composition or functional profiles under the conditions tested. These findings suggest that the compounds associated with the observed microbial responses were present in both substrate preparations rather than being restricted to the skin, contrary to the original hypothesis. Overall, these findings demonstrate that dietary substrate composition can influence both microbial community structure and functional potential, highlighting the importance of substrate characteristics in shaping gut microbiome responses.

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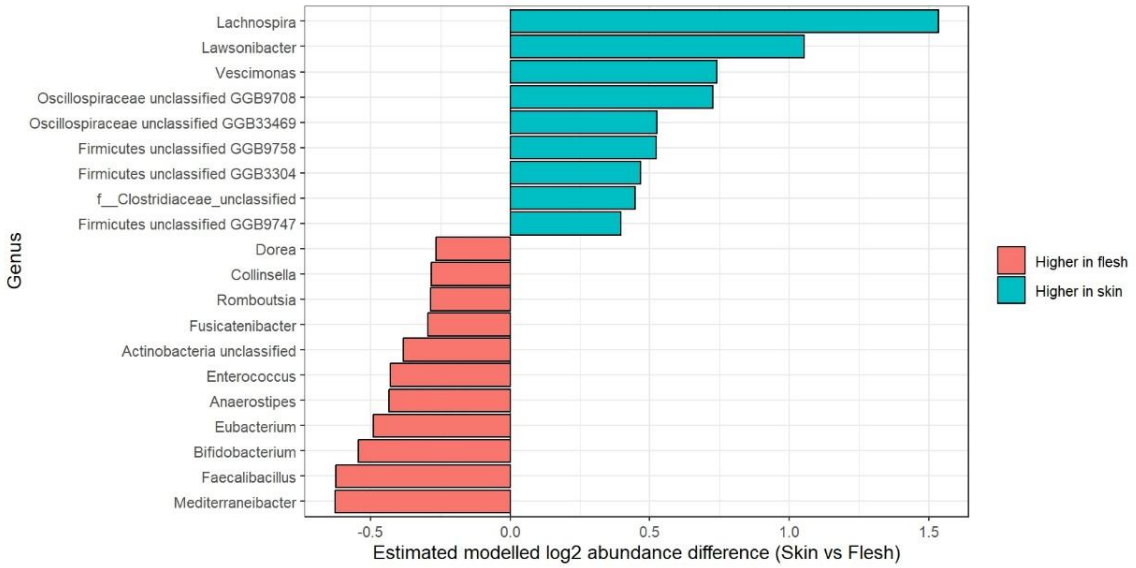
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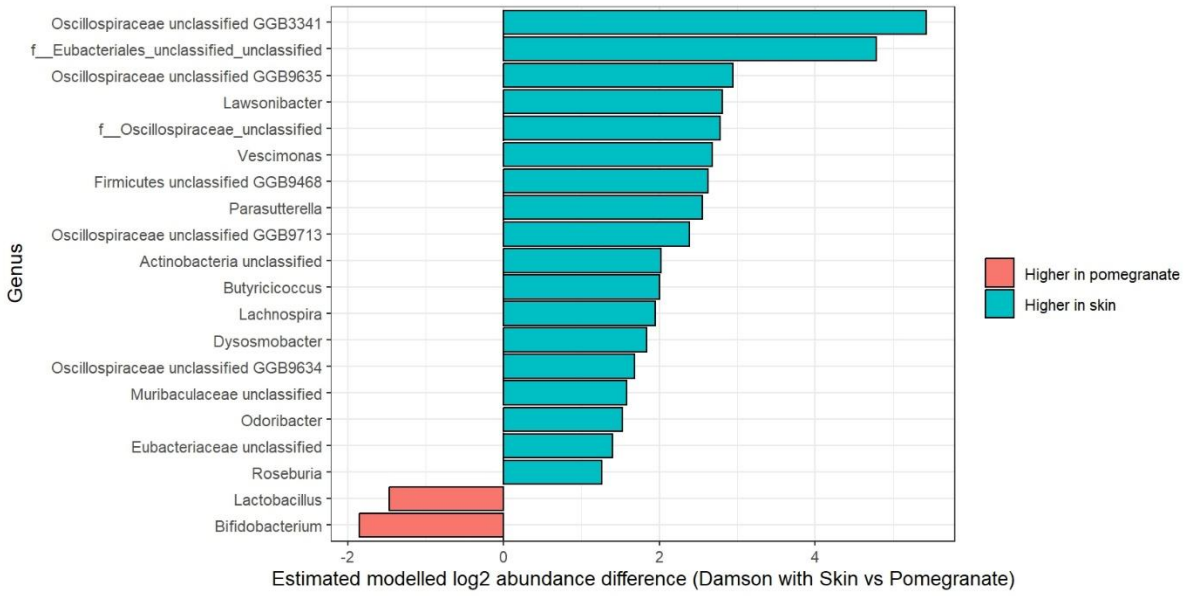
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Appendices

Appendix A. Supplementary figures



Supplementary Figure 1. Estimated differences in genus abundance between damson skin-on and damson flesh treatments. Values represent estimated log_2 abundance differences. Positive values indicate higher abundance in damson skin-on treatments relative to damson flesh, whereas negative values indicate higher abundance in damson flesh treatments. Only genera identified as significantly differentially abundant ($q < 0.1$) are shown.



Supplementary Figure 2. Differential abundance of genera in response to damson skin and pomegranate. Values are shown as estimated log_2 abundance differences. Positive values (blue) indicate higher abundance and negative values (red) indicate lower abundance relative to pomegranate. A log2 abundance change of +1 corresponds to a doubling in abundance, while −1 corresponds to a halving. Only genera identified as significantly differentially abundant ($q < 0.1$) are shown.

In-vitro fermentation with human colonic microbiota

Collection of stools (faecal material) for *in vitro* fermentation experiments to evaluate how nutrients in foods are fermented by gut microbes

SCREENING QUESTIONNAIRE

This screening questionnaire is to assess your suitability to take part in the study. Any information you provide is confidential to the researchers.

In keeping with ethical protocols, please note that you have the right to:

- not answer any particular question
- ask for more information about these questions at any time

Please answer the following questions.

Full Name: _____

Address: _____

_____

_____

Mobile Number: _____

Email: _____

Date of birth: _____ (dd/mm/yyyy)

Do any of the following apply to you?

- Smoker and/or recreational user of drugs
- Thyroid disorder
- Liver disorder (e.g. hepatitis)
- A history of or have kidney disorder (e.g. kidney stones)
- Diet-related illness (e.g. diabetes)
- A history of or have a gastrointestinal disorder (e.g. inflammatory bowel disease)
- Had gastrointestinal surgery in the past 6 months
- A history of or have digestive and/or bowel problems
- For women, you are pregnant or lactating or intending to be pregnant during the study

Yes ☐

If yes, this may make you ineligible to participate in the study.

No ☐ **Not Sure** ☐

Thank you for completing the questionnaire

Appendix C. Participant consent form

Riddet Institute (PN 445)

Massey University

Private Bag 11 222

In-vitro fermentation with human colonic microbiota

Collection of stools (faecal material) for *in vitro* fermentation experiments to evaluate how nutrients in foods are fermented by gut microbes

PARTICIPANT CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me.

My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet.

I wish to receive a summary of the results when available. Yes ☐ No ☐

My preferred method of contact for the results is by Email ☐

Mail ☐

Signature: ..... **Date:** .....

Full Name - printed .....

.....

Appendix D. PERMANOVA output generated using the adonis2 function in R

```
Damson skin on versus skin off

Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999

adonis2(formula = dist_sub ~ Type, data = meta_sub, permutations = 999)
         Df SumOfSqs    R2      F Pr(>F)
Model     1 0.031515 0.385 6.2603  0.006 **
Residual 10 0.050342 0.615
Total    11 0.081857 1.000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Pomegranate vs skin off (flesh)

Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999

adonis2(formula = dist_sub ~ Type, data = meta_sub, permutations = 999)
         Df SumOfSqs      R2     F Pr(>F)
Model     1  0.21172 0.50198 10.08  0.002 **
Residual 10  0.21005 0.49802
Total    11  0.42177 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


Pomegranate versus skin on

Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999

adonis2(formula = dist_sub ~ Type, data = meta_sub, permutations = 999)
         Df SumOfSqs      R2      F Pr(>F)
Model     1  0.28093 0.56144 12.802  0.004 **
Residual 10  0.21944 0.43856
Total    11  0.50037 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


Damson_flesh PotatoStarch
           6            6
>
> dist_sub <- as.dist(as.matrix(bc)[keep, keep])
>
> adonis2(
+     dist_sub ~ Type,
+     data = meta_sub,
+     permutations = 999
+ )
Permutation test for adonis under reduced model
```

```
Permutation: free
Number of permutations: 999

adonis2(formula = dist_sub ~ Type, data = meta_sub, permutations = 999)
         Df SumOfSqs      R2     F Pr(>F)
Model     1  0.47562 0.89284 83.32  0.001 ***
Residual 10  0.05708 0.10716
Total    11  0.53270 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> keep <- meta$Type %in% c("PotatoStarch", "Pomegranate")
>
> meta_sub <- droplevels(meta[keep, ])
>
> table(meta_sub$Type)

 Pomegranate PotatoStarch
           6            6
>
> dist_sub <- as.dist(as.matrix(bc)[keep, keep])
>
> adonis2(
+     dist_sub ~ Type,
+     data = meta_sub,
+     permutations = 999
+ )
Permutation test for adonis under reduced model
Permutation: free
Number of permutations: 999

adonis2(formula = dist_sub ~ Type, data = meta_sub, permutations = 999)
         Df SumOfSqs      R2      F Pr(>F)
Model     1  0.16651 0.42402 7.3616   0.02 *
Residual 10  0.22618 0.57598
Total    11  0.39269 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


dispersion test (data skewed?)
Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
          Df   Sum Sq  Mean Sq      F N.Perm Pr(>F)
Groups     3 0.035145 0.011715 2.2739    999  0.099 .
Residuals 20 0.103039 0.005152
---
```