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Valorisation of spent coffee grounds in the context of prebiotic potential: a review

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ABSTRACT

Spent coffee grounds (SCGs) are the solid residues generated after coffee brewing and have been widely researched due to their rich carbohydrate content. Beyond energy generation, there is a growing interest in developing functional food ingredients. This review focuses on assessing various extraction methods for SCG-derived compounds in terms of their prebiotic potential.

One common type of functional carbohydrate extracted from SCGs is mannooligosaccharides (MOS), primarily obtained through single-stage and enhanced extraction strategy. Single-stage extraction often uses one method, with integrated mechanism and yields oligosaccharides and monosaccharides. On the other hand, the enhanced extraction combines pre-treatments and enzymatic hydrolysis to increase the SCG extractability and selectively degrade polysaccharides. This method yields fewer undesired by-products and aims to avoid complete hydrolysis of SCG into monosaccharides.

In this review, several research gaps were identified in relation to fully valorise SCG. First, there is a critical gap in standardized analytical methods for accurately determining the profile of extracted oligosaccharides. Developing and adopting validated techniques is essential for a reliable characterization of these compounds. Second, the efficacy of pretreatment processes on SCG remains challenging to assess due to the lack of uniform evaluation criteria. Establishing such criteria would help compare across studies, ensuring more consistent assessment of pretreatment outcomes. Finally, the criteria for confirming prebiotic potential in SCG-derived compounds are not well understood. It is essential to adhere to established definitions of the term 'prebiotic' and to apply validated methodologies to assess their prebiotic status.

1. Introduction

Spent Coffee Grounds (SCGs) are generated by coffee shops, coffee retailers, as well as individuals (Johnson et al., 2022). The International Coffee Organization summarized that in year 2022/2023 global coffee consumption was around 173.1 million bags, equal to 10.39 million tonnes (Information Commissioner's Office, 2024). One tonne of green coffee yields nearly 650 kg SCG (Campos-Vega et al., 2015). From green coffee beans to commercial coffee drinks, coffee bean roasting is a key procedure as it not only partially changes coffee bean composition but also brings a certain aroma and flavour to coffee beverages (Carcea et al., 2023). SCG are commonly used for energy production such as pellets or biofuel, but research has also highlighted their potential health-promoting properties (Machado et al., 2023). Given their high abundance in carbohydrates, SCG are promising candidates for developing novel functional food ingredients, such as prebiotics (Bevilacqua et al., 2023). A prebiotic is a substance that beneficial microorganisms in the host utilize, resulting in health benefits for the host organism (Gibson et al., 2017; Hutkins et al., 2024). Notably, oligofructose (OF) and galactooligosaccharides (GOS), two well-researched prebiotics, are efficiently fermented by gut microbiota and influence its composition. The hydrolysis of SCG presents a valuable opportunity to expand the pool of candidate prebiotic substances. Despite SCG being an abundant waste biomass, the prebiotic potential of their derived compounds has not been systematically assessed, particularly in relation to the various extraction strategies employed. This review aims to systematically evaluate the valorisation methods applied to spent coffee grounds, with a particular emphasis on strategies that extract functional carbohydrates exhibiting prebiotic potential. It provides a comprehensive comparison of extraction techniques, analytical methodologies, and prebiotic assessment methods, ultimately outlining strategies for the optimal utilization of spent coffee grounds in functional food applications.

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2. SCG: a rich source of structural carbohydrates

SCG contain a considerate proportion of carbohydrates (Bevilacqua et al., 2023). As summarized in Table 1. The primary structural components of SCGs include cellulose, hemicellulose, and lignin. Hemicellulose is characterized as an amorphous, branched heteropolymer, consisting of hexoses and pentoses. SCG hemicellulose is particularly rich in galactomannans and arabinogalactans. Galactomannans are defined by a mannose backbone with linked galactose residues (Gorin et al., 1969), while arabinogalactan is constituted from arabinose and galactose monosaccharides (Daffe et al., 1990). Due to its amorphous structure, hemicellulose exhibits low crystallinity, which enhances its susceptibility to hydrolytic cleavage. In contrast, cellulose has a highly crystalline structure, rendering it more stable and resistant to conditions that readily degrade hemicellulose (Salem et al., 2023). Lignin is a complex, hydrophobic polymer that forms a protective barrier around cellulose and hemicellulose in plant cell walls, contributing to structural rigidity and resistance to degradation during enzymatic hydrolysis. In biorefinery processes aimed at producing fermentable sugars, improving the accessibility of cellulose is crucial for maximizing yield (Li et al., 2021; Yuan et al., 2021). Although producing functional oligosaccharides differs from extracting fermentable sugars, both methods rely on disrupting the cellulose and hemicellulose structure. In the case of oligosaccharide production, it is important to avoid the complete break down of these polymers, ensuring that the resulting products retain the desired degree of polymerization.

3. Extraction of SCG-derived hemicellulose and oligosaccharides

Hemicellulose typically accounts for approximately 20 %–35 % of the biomass in SCG (Zabaniotou & Kamaterou, 2019), with its proportion influenced by factors such as bean origin, roasting conditions, and brewing methods (Oosterveld et al., 2003). Although roasting slightly reduces the overall hemicellulose content, it also promotes depolymerization, thereby facilitating the release of simpler sugars that can be more efficiently extracted in subsequent processing stages (Redgwell et al., 2002; Zhao et al., 2024). Single-stage extraction methods play a crucial role in biomass valorisation, particularly when converting SCGs into value-added oligosaccharides and hemicellulose fractions (Table 2). These methods aim to degrade the cell wall matrix and release carbohydrate polymers with minimal processing steps. In the context of SCGs, they often operate under integrated mechanisms, combining thermal, chemical, and sometimes physiochemical processes. Common approaches include hydrothermal conversion, autohydrolysis, subcritical water extraction, and hot water extraction. These techniques share a key feature: they use high temperature and pressure to speed up the breakdown of structural carbohydrates while minimizing the use of chemicals.

Table 1
Presents the proximate chemical composition of SCG.

Chemical Component	Composition Range (wt %[a])	Reference
Moisture	58–74	Ballesteros et al. (2014)
Ash	0.6–4.7	Ballesteros et al. (2015)
Protein	10–19	Barampouti et al. (2022)
Lipid (Total fats)	2.3–18	Jiménez-Zamora et al. (2015)
Total Carbohydrate[b]	55–71.4	Vakalis et al. (2019)
Soluble Dietary Fibre (SDF)	2–9.7	Batista et al. (2020)
Insoluble Dietary Fibre (IDF)	35–50.7	López-Barrera et al. (2016)
Cellulose	7.6–22.2	Han et al. (2021)
Hemicellulose	30–42	Caballero-Galvan et al. (2018)
Lignin (Total)	20–24	Murthy and Naidu (2012)
Soluble lignin	17.6–27	
Klason lignin (Insoluble)	1–6.3	

[a] All values, except moisture content, are based on dry weight.
[b] Carbohydrates may include fibre, simple sugars, and polysaccharides.

Hydrothermal conversion is widely applied for hemicellulose breakdown and extraction, while preserving cellulose (Zhou et al., 2023). Ramos-Andrés et al. (2019) achieved a hemicellulose yield of 3.49 g/100 g of dry SCG using a flow-through reactor, but the yield was relatively low, likely due to lipid interference. Attenuated total reflectance-flourier transform infrared (ATR-FTIR) spectra suggested that polysaccharides were more concentrated after coffee oil was removed, indicating that lipid removal could enhance extraction efficiency.

In contrast, autohydrolysis, a specific type of hydrothermal treatment that employs endogenous water within the biomass, shows more promising results. Gu et al. (2020) combined autohydrolysis with enzymatic hydrolysis to extract galactomannan, achieving a 50.1 % yield, while Ballesteros et al. (2017) reported a total polysaccharide yield of 33 % at 160 °C in 10 min. Similarly, subcritical water extraction (SWE), which operates at temperatures between 100 °C and 374 °C, has shown efficacy in producing hemicellulose from SCGs (Mayanga-Torres et al., 2017; Vandeponseele et al., 2020). The method is conducted under temperatures and pressures below the critical point of water (Toor et al., 2011). It maintains water in its liquid state but increases its reactivity, allowing it to act as a catalyst for obtaining hemicellulose fractions. In comparison to both hydrothermal and autohydrolysis methods, SWE operates at higher pressures but lower temperatures. Pedras et al. (2019) performed SWE using a semi-continuous reactor, which facilitates the separation of different fractions over time. The study reported that the yield of carbohydrates (mainly hemicellulose) increased with temperature, peaking at around 33.7 % (w/w) per dry SCG at 200 °C. The extracted monosaccharides accounted for less than 5 % of the total carbohydrates, with arabinose being the most abundant, followed by mannose and galactose. The low yield of glucose in the extracts suggests that cellulose largely stayed intact during the process. This also matches to the recalcitrant nature of the cellulose, as the temperatures used were insufficient to extensively hydrolyse cellulose.

de Cosío-Barrón et al. (2020) and Tian et al. (2017) aimed at producing mannooligosaccharides (MOS) by applying hot water extraction. While no comprehensive yield data of specific oligosaccharides were provided from both studies to evaluate the application of hot water extraction in SCG valorisation, authors suggested that the extracted oligosaccharides exhibited functional properties.

Asano et al. (2003) and Perez-Burillo et al. (2019) utilized autohydrolysis to extract MOS from SCG. The first study reported MOS, and monosaccharide yields of 29 % (w/w) per dry SCG, whereas later achieved a yield of 4.135 % (w/w) MOS per dry SCG. It is obvious that MOS yields differ vastly from each other. Asano et al. (2003) used high-pressure steam in a plug flow reactor with a much shorter residence time (8 min), which is considered a more aggressive process. Fabrizio et al. (2021) utilized acid hydrolysis at high temperatures (200 °C), and the hydrolysates obtained were shown to consist mainly of oligosaccharides with a degree of polymerization between 3 and 6. The yield of specific oligosaccharides was not provided, limiting direct comparison.

In general, hydrothermal conversion has been widely used not only for SCG hemicellulose production but also for functional oligosaccharides. With the use of reactors, these extraction strategies may be suitable for large-scale biomass valorisation. However, they are energy-intensive and time-consuming; more recent advances in extraction technologies have shifted toward methods that offer faster and more energy-efficient alternatives. One such method is microwave-assisted extraction (MAE), which, like hydrothermal conversion, employs heat to facilitate the breakdown of structural carbohydrates. MAE uses microwave energy to heat solvents in contact with a sample, promoting the transfer of target compounds from the sample matrix into the solvent, leading to more localized and rapid heating (Sparr Eskilsson & Björklund, 2000). The interaction between microwaves and biomass

Table 2
Extraction processes and yields of hemicellulose and hemicellulose degraded oligosaccharide from Spent Coffee Grounds (SCG).

Extraction Method	Chemical Solvent	Temperature and Time (Pressure)	Oligosaccharides/Hemicellulose (wt%)[a]	Reference
Hot water extraction	None	100 °C, 20 min 80 °C, 60 min	Not quantified Not quantified	Tian et al. (2017) de Cosío-Barrón et al. (2020)
Hydrothermal extraction	None	140–160 °C, 0–40 min	Hemicellulose: 3.49	Ramos-Andrés et al. (2019)
Autohydrolysis	None	120–160 °C, 60 min 220 °C, 8 min (Steam pressure) 170–220 °C, 10–60 min	Galactomannan: 50.1 MOS: 33 MOS: 4.135	Gu et al. (2020) Asano et al. (2003) Perez-Burillo et al. (2019)
Subcritical water extraction	None	150–220 °C, 30 min (70 bar)	Total carbohydrates: 33.7	Pedras et al. (2019)
Microwave-assisted extraction	Diluted 0.1M NaOH and aqueous	140–220 °C, 2–10 min	Total carbohydrates: 2.9 Galactomannan: 1.4 (43 wt% per total carbohydrates) Arabinogalactan: 1.45 (57 wt% per total carbohydrates)	Passos et al. (2019)
Acid-catalysed hydrolysis	HCl 5 % (w/v) NaCl and acetic acid	200 °C, 0.5–1.5 min	trisaccharides (>70)	Fabrizio et al. (2021)

[a] All optimal yield, unless stated, the yield is calculated on dry weight SCG.

accelerates the extraction process, reducing energy consumption while preserving the structural integrity of the target hemicellulose fractions. Passos et al. (2019) extracted arabinogalactans and galactomannans from SCG. The maximum yield for galactomannans and arabinogalactans at 170 °C was around 39 g kg^{-1} and 110 g kg^{-1} dry SCG, respectively, with the addition of 0.1 M NaOH solution. At temperatures higher than 170 °C, degradation of polysaccharides, particularly arabinogalactans, was observed. These oligosaccharides yields were relatively low compared to subcritical water extraction but higher than autohydrolysis.

4. Enhanced extraction strategies: pre-treatment for oligosaccharide production from SCG

Although one-step conversion is applied in producing functional oligosaccharides from SCG, pre-treatment followed by enzymatic hydrolysis can enhance the yield of oligosaccharides. Traditionally, pre-treatment processes have been used widely on SCG biorefinery processes, aiming at fermentable sugars for biofuel production (Jin et al., 2020; Lee et al., 2021; Titiri et al., 2023). However, in the context of producing functional oligosaccharides from SCG, certain factors need special consideration. These include high recovery of hemicellulosic sugars to boost enzymatic hydrolysis; minimization of toxic compounds such as furfural and hydroxymethylfurfural (HMF), which can be formed through sugar degradation; and optimal process conditions to avoid unnecessary degradation of targeted oligosaccharides (Alvira et al., 2010; Kumar & Sharma, 2017; Zhang et al., 2021). Pre-treatment strategies can be classified into chemical, physicochemical, and combinations of these methods. Fig. 1 summarises the workflow of the extraction and prebiotic potential of the functional carbohydrates from SCG.

4.1. Chemical pre-treatment

Chemical pre-treatment utilizes alkaline or acidic reagents to partially cleave the structure of SCG, enhancing the accessibility of polysaccharides, such as cellulose and hemicellulose. Its efficacy is primarily assessed by quantifying the polysaccharide fractions remaining in the pre-treated SCG, which indicates their accessibility for further enzymatic hydrolysis. In addition, analysis of the liquid extract for solubilized sugars (oligosaccharides or monosaccharides) can help the evaluation of the severity of the pre-treatment. Among NaOH pre-treatments, the conditions used by Ibrahim et al. (2022) and Jin Cho et al. (2022) both demonstrated significant hemicellulose recovery, though the latter applied a milder NaOH concentration at a higher temperature (1M NaOH at 80 °C) and increased the galactomannan content from 348 g kg^{-1} to 365 g kg^{-1} per dry SCG. Wongsiridetchai et al. (2018) investigated the effect of NaOH concentration on SCG pre-treatment efficacy, by optimising conditions such as substrate-to-liquid ratio and temperature. Under optimised conditions, reducing sugar yield was equal to 10.5 % (w/w) per dry SCG. Furthermore, scanning electron microscope (SEM) analysis indicated that temperatures exceeding 100 °C can lead to excessive hemicellulose degradation, while thin-layer chromatography (TLC) showed that the

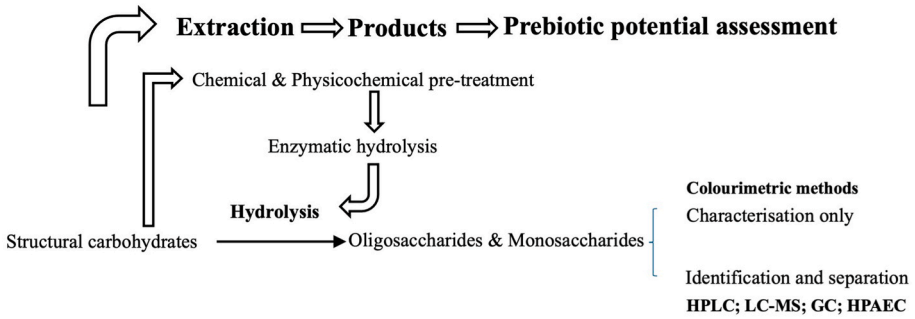

Fig. 1. Summary workflow of extraction and prebiotic potential assessment of functional carbohydrates from SCG.

obtained hydrolysates contained mannobiose and mannotriose. While Magengelele et al. (2023) and Jin Cho et al. (2022) used thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR) to confirm effective lignin removal, the lack of direct lignin quantification in their analyses highlights a limitation of current methodologies. Future studies should incorporate quantitative techniques such as acid detergent lignin (ADL) analysis to provide solid evidence in lignin removal.

In contrast, diluted acid pre-treatment is less frequently applied due to its tendency to degrade polysaccharides. Hemicellulose, with its low crystallinity, is more susceptible to acid hydrolysis than cellulose, which has strong β-1,4-glycosidic bonds (Lorenci Woiciechowski et al., 2020). Thus, diluted acid pre-treatment can selectively degrade hemicellulose, often leading to the formation of inhibitory by-products.

Quynh Anh et al. (2019) reported a significant increase in mannose of the pre-treated SCG, from 19.3 % (w/w) to 58.2 % (w/w) per dry SCG, along with a sharp reduction in lignin content, from 38.6 % (w/w) to 2.3 % (w/w) per dry SCG. Although the increase of mannose remained unclear, it is obvious that diluted acid pre-treatment can increase mannose content in the liquid extract, therefore less mannan could stay intact in the biomass. Conversely, Ravindran et al. (2017) observed a decrease in mannan content, from 21.1 % (w/w) to 5.8 % (w/w) per dry SCG, accompanied by an increase in cellulose content, from 8.6 % (w/w) to 15.37 % (w/w). Notably, the total lignin content in Ravindran et al. (2017) exhibited only a modest decrease, from 30 % to 27 % (w/w). The difference between these two studies can be attributed to differences in the acid concentration, pre-treatment temperature, and duration. For example, Ravindran et al. (2017) employed 1.6 % (v/v) sulfuric acid at 121 °C for 20 min, conditions that facilitated the formation of furfural and HMF. These results suggest that diluted acid pre-treatment, while effective in some contexts, may not be the most suitable approach for enhancing hemicellulose accessibility in SCG, particularly due to its tendency to generate inhibitory by-products and break down hemicellulosic sugars.

Other chemical pre-treatment strategies have also been explored, such as ferric chloride ($FeCl_3$). Ferric chloride acts by disrupting the lignin-polysaccharide linkages and loosening the cell wall structure (Chen et al., 2015). Ravindran et al. (2017) demonstrated that $FeCl_3$ treatment reduced acid-insoluble lignin (AIL) content from 27.12 % (w/w) to 12.32 % (w/w) per dry SCG, while largely preserving the cellulose and mannan fractions. Despite these promising results, the use of ferric chloride necessitates the recovery of metal ions, which adds an additional step and potential cost to the process (Awasthi et al., 2022).

Alkaline hydrogen peroxide (AHP) has emerged as another promising pre-treatment method. Jin Cho et al. (2022) reported significant improvements in both mannan and cellulose recovery, with mannan increasing from 22.61 % (w/w) to 30.38 % (w/w) and cellulose from 11.40 % (w/w) to 20.59 % (w/w) per dry SCG. The oxidative nature of H_2O_2 facilitates the generation of hydroxyl radicals (-OH), which effectively break down lignin, thereby enhancing subsequent enzymatic hydrolysis (Dutra et al., 2018). However, the use of AHP introduces challenges as it is a time-consuming process and requires multiple preliminary tests to confirm the appropriate concentration range of H_2O_2. Excessive gas could be formed and cause overflow and foaming. Organosolv pre-treatment represents a more complex approach that employs organic solvents such as acetone, methanol, ethanol, or ethylene glycol in conjunction with water. This method effectively solubilizes hemicellulose and detaches lignin, significantly enhancing the enzymatic hydrolysis of cellulose (Anu et al., 2020). Ravindran et al. (2017) observed an increase in mannan content, from 21.1 % (w/w) to 31.54 % (w/w) in SCG biomass. While this method shows great potential, it is accompanied by operational challenges such as solvent recovery and disposal, as well as safety concerns associated with the use of volatile organic compounds.

4.2. Physicochemical pretreatment

Physicochemical pre-treatments such as steam explosion, microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE), integrate both physical and chemical processes to enhance oligosaccharide yields. MAE accelerates extraction by selectively heating polar molecules, significantly reducing extraction time and solvent usage (Destandau & Michel, 2022). MAE promotes cell wall disruption, improving polysaccharide accessibility for subsequent hydrolysis (Sparr Eskilsson & Björklund, 2000). UAE enhances cell wall disruption in SCG by increasing mass transfer efficiency through cavitation bubbles. As these bubbles collapse near plant material, they generate microjets that break down cell walls, improving access to SCG carbohydrate fractions for hydrolysis.

These techniques have been applied in the pre-treatment stage for SCG valorisation. Studies by Getachew and Chun (2017) and Getachew et al. (2018) demonstrated that MAE and UAE improved yields in reducing sugars, suggesting enhanced polysaccharide recovery. However, while the studies indicated increased sugar production, they did not specifically report on the yields of functional oligosaccharides or detailed carbohydrate fraction content; it is also unknown that how these pre-treatments can help increase the accessibility of the hemicellulose fraction in SCG.

Steam explosion is a widely applied physicochemical pre-treatment technique for lignocellulosic biomass, including SCG. This process involves subjecting SCG to saturated steam under high pressure for a short period, followed by a rapid decompression. The sudden pressure drop disrupts and opens the polysaccharide matrix, enhancing the accessibility of cellulose and hemicellulose for further hydrolysis (Chandra et al., 2015; Ziegler-Devin et al., 2021). In particular, the process solubilizes hemicellulose into water-soluble fractions, thereby increasing the recovery of cellulose to produce fermentable sugars.

Given the nature of steam explosion, both the liquid extract and the water-insoluble solid residue must be analysed post-treatment to evaluate the degree of solubilization and recovery. Monitoring these fractions is critical for understanding the extent of hemicellulose solubilization and cellulose preservation, which directly affects the yield of valuable carbohydrates. For example, Chiyanzu et al. (2014) reported that steam explosion conducted at lower temperatures (150 °C) resulted in no detectable oligomeric sugars in the liquid extract, while glucan and mannan contents in the solid residue slightly decreased, from 24.17 g/100 g SCG to 22.14 g/100 g SCG, and 24.67 g/100 g SCG to 21.38 g/100 g SCG, respectively. Additionally, longer reaction times and higher temperatures can lead to extensive breakdown of mannan into MOS in the liquid extract. Chiyanzu et al. (2015) reported a massive reduction in mannan content when operating at 210 °C for 15 min, from 24.67 g/100 g SCG to 18.16 g/100g SCG.

While steam explosion may not be the best pre-treatment method in elevating the hemicellulose fraction in SCG, it can help loosen up the cell polysaccharide structure. The results also showed that this method is unable to remove lignin as the Fourier-transform infrared spectroscopy (FT-IR), sampling with attenuated total reflectance (ATR) analysis showed an increase in lignin component. This does not imply that this method is not efficient at all, as the study indicated that by using the steam explosion, it can reduce enzyme loading with an increased enzyme digestibility.

It is crucial to evaluate the efficiency of pre-treatments. To enhance the accessibility of hemicellulose and to increase the yield of functional oligosaccharides, typical goals of pre-treatments should be: (1) avoid SCG hemicellulose fraction degradation; (2) reduce formation of any inhibitors or by-products for further enzymatic hydrolysis; (3) removal of lignin content; and (4) production of highly digestible pre-treated SCG that improves the oligosaccharide yield during enzymatic hydrolysis. Ravindran et al. (2017) applied eight different pre-treatment methods to establish an effective pre-treatment SCG strategy. The study combined two chemical pretreatments as a sequential pre-treatment, using

concentrated phosphoric acid, ice-cold acetone, and ammonia. Sequential pre-treatment significantly reduced total lignin content from 31 % (w/w) to 11 % (w/w) per dry SCG, while enhancing cellulose content from 8.60 % (w/w) to 20.0 % (w/w) per dry SCG.

4.3. Enzymatic hydrolysis for oligosaccharide production

With the combination of optimised pre-treatment methods, enzymatic hydrolysis further breaks down polysaccharides into smaller oligosaccharides, such as mannooligosaccharides (MOS). In SCG, this process targets galactomannans, the predominant polysaccharide in coffee, to yield MOS and other fermentable sugars (Kumar Awasthi et al., 2022; Kumar et al., 2022; Wahlström & Suurnäkki, 2015). Enzymatic hydrolysis or synthesis pathways can generate functional oligosaccharides from various carbohydrate-rich biomass sources (Rastall, 2010; Yang et al., 2023). SCG are particularly rich in galactomannans, comprising approximately 50 % of the total polysaccharide content in green coffee beans (Moreira et al., 2015). These galactomannans feature a linear β-(1 → 4)-linked D-mannose (Man) backbone with α-D-galactose (Gal) side chains (Jana et al., 2021). Table 3 provides a comprehensive comparison of untreated SCG with various pretreatment methods, detailing their specific conditions, and subsequent enzymatic hydrolysis yields, highlighting the synergistic effects of combining pre-treatment and enzymatic hydrolysis.

The roasting of coffee beans enhances the extractability of galactomannans, although a significant portion remains insoluble within the SCG matrix (Moreira et al., 2011), showing the necessity of pre-treatment to improve substrate accessibility for enzymatic hydrolysis. The enzymatic hydrolysis of SCG primarily relies on β-1, 4-D-mannan mannohydrolase (β-mannanase, EC 3.2.1.78), which specifically hydrolyses β-1,4-mannosidic linkages in the mannan backbone (Hlalukana et al., 2021). Mannanase can be sourced from microbial (bacterial and fungal), plant, and animal origins, with the source influencing its hydrolytic efficacy on coffee mannan due to variations in enzyme structure, specificity, and optimal conditions (Malgas et al., 2015; Álvarez et al., 2016).

Bacterial mannanases, commonly derived from *Bacillus*, *Streptomyces*, and *Thermobifida*, are favoured for coffee mannan degradation due to their stability across broad pH and temperature ranges. Specifically, mannanase from *Bacillus* spp. demonstrates high thermal stability and efficacy in alkaline conditions, making it suitable for SCG galactomannan degradation (Dhawan & Kaur, 2007). For instance, an endo-β-1,4-mannanase (Man26A) from *Bacillus* sp. produced 2.47 mg/mL of MOS per hydrolysate at 50 °C, pH 7.0 over 48 h (Magengelele et al., 2023), though this yield could be improved by adjusting operational conditions.

In contrast, *P. purpurogenum* mannanase achieved the highest MOS yield of 43.3 % (w/w) per g of SCG at 50 °C, pH 5.0 for 48 h (Jin Cho et al., 2022). Similarly, a β-mannanase from *Aureobasidium pullulans* NRRL 58524 yielded 58.22 ± 2.04 mg per 100 mg of pre-treated SCG at 55 °C, pH 4.0 over 41 h, demonstrating significant yield over an extended period (Ibrahim et al., 2022). Combining enzymes can enhance yield by targeting different polysaccharide components, thus improving hydrolysis efficiency (Agrawal et al., 2018; Contreras et al., 2020). A combination of endo-1,4-β-D-mannanase and cellulase from *Acremonium* sp. yielded 57.79 % (w/w) MOS and 22.38 % (w/w) residual mannan at 60 °C, pH 4.8 for 18 h, indicating the potential of enzyme blends (Chiyanzu et al., 2014, 2015). Notably, fungal mannanases operate better under acidic conditions, while bacterial mannanases are more effective in neutral environments.

Commercial cellulase Celluclast®, derived from *T. reesei* and *T. longibrachiatum*, combined with in-house produced pectinase and cellulase, yielded 72.1 mg/mL of MOS at 45 °C, pH 4.8 over 12 h (Quynh Anh et al., 2019). These findings highlight variability in yield and efficiency based on enzyme source, with tailored solutions available for industrial applications depending on required conditions.

Table 3
Enhanced extraction strategies of oligosaccharides and hemicellulose from SCG.

Pre-treatment	Enzymatic Hydrolysis Conditions	Yield (wt%)[a]	Reference
NaOH pre-treatment 50–121 °C/2–96 h/ 1:1–1:5	Mannanase (*Bacillus* sp. GA2 (1)) 50 °C 5 h pH 6.0	Reducing sugar: 10.525 wt%	Wongsiridetchai et al. (2018) Wongsiridetchai et al. (2021) Puengsawad et al. (2021)
NaOH pre-treatment 37 °C/24 h/ 1g:10 ml	β-mannanase from *Aureobasidium pullulans* NRRL 58524 55 °C 41 h pH 4.0	Galactomannan: 54 wt% per SCG hemicellulose	Ibrahim et al. (2022)
NaOH pre-treatment H_2O_2 – NaOH pre-treatment 80 °C/1 and 2 h/1g:10 ml	Mannanase from *P. purpurogenum* 50 °C 48 h pH 5.0	MOS: 43.3 wt%	Jin Cho et al. (2022)
NaOH pre-treatment 70 °C/4 h/ 1g:20 ml	*Bacillus* sp. derived endo-β-1,4-mannanase, Man26A 50 °C 48 h pH 7.0	MOS: 2.47 mg/mL per enzymatic hydrolysate liquid	Magengelele et al. (2023)
Microwave-assisted NaOH pre-treatment 800W/30 s/ 1g:10 ml (1 % NaOH)	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase 50 °C/24 h/pH 6.8	Cellulose:11.02 wt % per raw SCG Hemicellulose (Galactan and mannan): 14.78 wt % per raw SCG Reducing sugar: 25.8 wt% per pre-treated SCG	Ravindran et al. (2017)
Diluted acetic acid pre-treatment 80 °C/3 h/ 1g:10 ml	Commercial cellulase: Celluclast® Cellulase from *T. reesei* and *T. longibrachiatum* In-house produced pectinase and cellulase *(without specifying the origin)* 45 °C 12 h pH 4.8	Total carbohydrates: 41.5 wt % MOS: mainly mannobiose (24.5 wt%) and mannohexaose (19.8 wt%) per SCG total carbohydrates Mannose: around 49 wt% per SCG total carbohydrates	Quynh Anh et al. (2019)
Modified diluted acid hydrolysis 1 %, 1.3 %, 1.6 % (v/v) H_2SO_4 121 °C/10–30 min/1g:10 ml	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase 50 °C/24 h/pH 6.8	Cellulose:15.37 wt % per raw SCG Hemicellulose (Galactan and mannan): 9.72 wt % per raw SCG Reducing sugar: 26.4 wt% per pre-treated SCG	Ravindran et al. (2017)
Concentrated phosphoric acid 50 °C/1 h/ 1g:10 ml (85 % H_3PO_4)	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase	Cellulose:18.14 wt % per raw SCG Hemicellulose (Galactan and mannan): 7.15 wt % per raw SCG	Ravindran et al. (2017)

(*continued on next page*)

Table 3 (*continued*)

Pre-treatment	Enzymatic Hydrolysis Conditions	Yield (wt%)[a]	Reference
	50 °C/24 h/pH 6.8	Reducing sugar: 28.3 wt% per pre-treated SCG	
Steam explosion	*Endo*-1,4-β-D-mannanase & Cellulase (*Acremonium* sp.)	Total carbohydrate: 27.65 wt%	Chiyanzu et al. (2014)
Saturated steam (30 bars) 121–200 °C/10–30 min 2.5 MPa steam explosion reactor	60 °C 18 h pH 4.8	Reducing sugar: 22.6 wt% per untreated SCG MOS: 57.79 wt% per pre-treated SCG mannan Mannan: 18.16 wt % per pre-treated SCG	Chiyanzu et al. (2015)
Steam explosion 2.5 MPa steam explosion reactor 121 °C/30 min/ 50 % moisture content	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase	Cellulose:6.20 wt% per raw SCG Hemicellulose (Galactan and mannan): 15.5 wt % per raw SCG	Ravindran et al. (2017)
	50 °C/24 h/pH 6.8	Reducing sugar: 25.9 wt% per pre-treated SCG	
Ammonia fibre explosion (AFEX) pre-treatment 120 °C/30 min/ 1g:10 ml (NH_4OH)	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase	Cellulose: 8.70 wt % per raw SCG Hemicellulose (Galactan and mannan): 15.02 wt % per raw SCG	
	50 °C/24 h/pH 6.8	Reducing sugar: 27.5 wt% per pre-treated SCG	
Ferric chloride pre-treatment 120 °C/30 min/ 1g:50 ml (0.1 M $FeCl_3$)	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase	Cellulose:10.74 wt % per raw SCG Hemicellulose (Galactan and mannan): 27.75 wt % per raw SCG	
	50 °C/24 h/pH 6.8	Reducing sugar: 27.5 wt% per pre-treated SCG	
Organosolv pre-treatment 120 °C/30 min/ 1g:25 ml (1 % H_2SO_4 in 50–70 % ethanol)	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase	Cellulose:7.04 wt% per raw SCG Hemicellulose (Galactan and mannan): 30.12 wt % per raw SCG	
	50 °C/24 h/pH 6.8	Reducing sugar: 28.3 wt% per pre-treated SCG	
Atmospheric plasma 4 min/80 kV/ 50 g SCG in polyethylene tray	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase	Cellulose:12.65 wt % per raw SCG Hemicellulose (Galactan and mannan): 18.77 wt % per raw SCG	
	50 °C/24 h/pH 6.8	Reducing sugar: 26.9 wt% per pre-treated SCG	
Sequential pre-treatment Phosphoric acid: 1 h, 50 °C, 1g:10 ml (85 % H_3PO_4) + AFEX: 30 min, 120 °C, 1g:10 ml	1.5 % (v/v) of cellulase 0.37 % (v/v) hemicellulase	Cellulose:20.01 wt % per raw SCG Hemicellulose (Galactan and mannan): 6.15 wt % per raw SCG	
	50 °C/24 h/pH 6.8	Reducing sugar: 35.0 wt% per pre-treated SCG	

[a] Unless stated, optimal yield is calculated on dry SCG weight basis.

While the production of MOS from SCG is well-researched due to the rich nature of galactomannans, other functional oligosaccharides such as galacto-oligosaccharides (GOS) and cellooligosaccharides (COS) remain underexplored. SCG contains around 10 % cellulose, yet studies have primarily focused on MOS extraction, leaving these potentially valuable oligosaccharides underutilized.

5. Identification and characterisation of SCG-derived compounds

Compositional and structural characterisation of oligosaccharides and hemicellulose derived from spent coffee grounds (SCG) is essential for evaluating the efficiency of extraction methods and ensuring the functionality of these biopolymers. A wide array of analytical techniques has been employed, including high-resolution mass spectrometry (HRMS), gas chromatography-flame ionization detection (GC-FID), thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Each of these techniques offers unique strengths and weaknesses, making a combination of approaches necessary for comprehensive analysis.

HRMS is highly effective in distinguishing oligosaccharides by their degree of polymerization. Fabrizio et al. (2021) utilized HRMS to identify oligosaccharides with polymerization degrees ranging from 3 to 6, though the method fell short in fully characterizing the oligosaccharide types. Similarly, Nano Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (NanoLC-QToF MS) has been employed (Tian et al., 2017) for precise mass measurements. However, the lack of commercial standards for certain oligosaccharides has hindered the quantification of individual oligosaccharides, stressing the need for standardized reference compounds in oligosaccharide analysis.

GC-FID is another prevalent method, particularly for monosaccharide profiling, capable of identifying sugars such as glucose, mannose, and galactose. However, it is limited in scope when applied to larger oligosaccharides, necessitating the use of additional techniques like HPLC to offer detailed oligosaccharide profiles. Even with HPLC, limitations arise when using inappropriate standards. For instance, de Cosío-Barrón et al. (2020) used raffinose, stachyose, and verbascose standards, compounds not naturally present in SCG, to quantify galactooligosaccharides (GOS), raising concerns about the reliability of results. Future analytical work must prioritize selecting appropriate standards and advanced chromatographic methods such as hydrophilic interaction liquid chromatography (HILIC) paired with pulsed amperometric detection (PAD) or evaporative light scattering detector (ELSD) to enhance accuracy and reproducibility (Li et al., 2016; Rodríguez-Gómez et al., 2015).

TLC, though commonly used for the qualitative analysis of mannooligosaccharides (MOS) due to its low cost and simplicity, is limited by its inability to provide quantitative data on individual MOS components. Similarly, the dinitrosalicylic acid (DNS) reducing sugar assay, while useful for measuring total reducing sugars, fails to differentiate between specific sugar types or provide data on MOS yields. Anthrone-sulfuric acid assays also face similar challenges in terms of specificity. As

a result, while these techniques are valuable for rapid assessments, they are inadequate for precise characterization of SCG-derived oligosaccharides.

The challenges of fully characterizing SCG-derived MOS are compounded by the diversity of oligosaccharides produced during enzymatic hydrolysis. More sophisticated techniques, such as HPLC combined with nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry, could provide the structural and quantitative insights necessary to refine extraction and processing methods. Bhaturiwala and Modi (2020) and Getachew et al. (2018) both emphasize the importance of integrating these high-resolution methods to optimize oligosaccharide production and characterization.

In addition to oligosaccharides, SCG's cellulose fractions offer potential for the extraction of cello-oligosaccharides (COS), particularly following enzymatic hydrolysis. With up to 20 % cellulose recovery reported post-pre-treatment (Ravindran et al., 2017), future research could explore COS alongside MOS as valuable bioactive compounds with applications in functional foods (Jin Cho et al., 2022).

6. Evaluation of prebiotic potential in spent coffee ground

6.1. Prebiotic potential assessment

Prebiotics are defined as substrates that are selectively metabolized by host microorganisms to confer health benefits and modulate the microbiota in both humans and animals (Gibson et al., 2017). Therefore, to verify the prebiotic status of oligosaccharides, it is essential to demonstrate both selective fermentation and the associated health benefits (Hutkins et al., 2024). Given the diversity of methodologies used to assess selective fermentation and related health outcomes, most studies can only demonstrate the potential for prebiotic activity. As such, while current definitions require robust evidence to classify an ingredient as a true prebiotic, we refer to these functional carbohydrates as possessing 'prebiotic potential' until comprehensive and statistically significant validation is achieved.

In vitro fermentation models are often used to evaluate fermentation, using a faecal inoculum to simulate the gut microbiota ecosystem more systematically (Pham & Mohajeri, 2018). Compared to pure culture studies, this approach provides a broader interpretation of microbial interactions. Batch fermentation models, which are suitable for short-term experiments, have limitations, such as rapid substrate depletion and pH reduction that can inhibit microbial activity (Pérez-Burillo et al., 2021). To improve the fermentation effectiveness, *in vitro* pH-controlled batch fermentation models have been developed, allowing for pH control in real-time during fermentation, ensuring microbial activity is not adversely affected by pH changes (Isenring et al., 2023). However, batch fermentation is not ideal for observing long-term changes, such as the accumulation of short-chain fatty acids (SCFAs) in the fermentation broth. High concentrations of these can inhibit microbial growth and fermentation performance, leading to reduced yields and productivity. Continuous fermentation models can offer a better simulation of gut fermentation processes by maintaining more stable conditions over time, which is important for observing prolonged microbial interactions (Moon et al., 2016).

Some studies also incorporate an *in vitro* digestion phase before fermentation, using static or dynamic models, aiming to test whether a candidate prebiotic or food can survive the oral, gastric, and intestinal digestive phases (Brodkorb et al., 2019). Static digestion models are easier to replicate and control but fail to simulate the dynamic, continuous flow of digestive fluids in the gastrointestinal system (Wang et al., 2021). The model overlooks susceptibility to small intestinal brush border (BB) glycosidases and selective absorption of monosaccharides into circulation or not incorporate the entire array of brush border enzymes necessary for the final stage of digestion in the body (Picariello et al., 2015). Therefore, the ability of a candidate prebiotic to survive enzyme reaction, cannot indicate its true digestibility. In contrast, dynamic digestion models offer a more accurate reflection of human physiology, controlling factors like enzyme concentration and pH changes (Ji et al., 2022). While more complex, these models provide better insights into the digestibility of potential prebiotics (Dupont et al., 2019).

In vivo trials, which directly examine the effects of prebiotics on gut microbiota in human or animal subjects, are often considered the gold standard. The most common study design is the placebo-controlled randomized trial, though crossover and parallel designs are also used (Bell et al., 2022; Gibson & Fuller, 2000; Tandon et al., 2019; Walton et al., 2012). The group of human volunteers recruited can vary depending on the study's objectives. For example, studies may investigate how prebiotics affect patients with irritable bowel syndrome (IBS) to assess potential health benefits (Wilson et al., 2019), how prebiotics can impact the mood state when participants are under mild/moderately increased level of anxiety and stress (Jackson et al., 2023), or how prebiotics can improve functional diarrhoea in children group (Du et al., 2023). These trials are essential for confirming prebiotic potential, as they provide evidence of measurable health benefits. Establishing such benefits is complex, requiring large amounts of food-grade materials and large-scale studies with statistically significant numbers of participants. Health benefits, known as beneficial physiological effect, must be specific, measurable, and distinct from mere microbiome changes, which alone do not constitute a health benefit (European Food Safety Authority, 2015).

Since measuring directly associated health benefits in human trials are difficult to achieve, the production of SCFAs such as acetate, propionate, and butyrate, is often used as an indirect indicator of prebiotic potential (Roberfroid et al., 2010; Sarbini & Rastall, 2011). However, Verbeke et al. (2015) pointed out that changes in faecal SCFA levels alone are not sufficient to confirm prebiotic efficacy.

Understanding changes in the gut microbiome is also key to assessing prebiotic potential. In healthy individuals, predominant bacterial genera are *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Lactobacillus Streptococcus* and enterobacteria in the human gut (Hou et al., 2022). For this reason, studies frequently focus on quantifying *Bifidobacterium* and *Lactobacillus* populations when assessing prebiotic potential. It is important to choose the right technique to identify and classify bacteria. Culture-based techniques are first applied on specific gut bacteria, such as *Bifidobacterium* and *Lactobacillus* (Mitmesser & Combs, 2017). While these methods effectively quantify target bacteria, they fail to capture the broader microbial interactions in the gut (Anadón et al., 2016; Davis, 2014). Molecular methods are most applied to analyse the stool microbiota (Franco-Duarte et al., 2019). Combined analysis methods such as metabolomics together with metagenomics are better at interpreting the prebiotic-induced microbiome modulation (Puig-Castellví et al., 2023).

6.2. Digestibility and fermentation of SCG-derived materials: MOS

Given the complexity of assessing prebiotic potential, the evaluation of SCG-derived materials, such as mannooligosaccharides (MOS), is resource-intensive. The techniques used for digestibility and fermentation assessments are summarized in Table 4.

As previously mentioned, cultural enumeration methods are not considered suitable for confirming prebiotic potential. However, several studies, including those by Puengsawad et al. (2021), Fabrizio et al. (2021), Wongsiridetchai et al. (2021), Magengelele et al. (2023), and Gu et al. (2020), utilized this approach to assess SCG-derived MOS. Montemurro et al. (2024) also evaluated the SCG-derived oligosaccharides as a growth substrate for probiotic lactic acid bacteria. Among 11 strains tested, *Lactiplantibacillus plantarum* LP19 showed superior growth (≈9.2 $\log_{10}$ CFU/g) on treated SCG, indicating the potential for synbiotic formulations. The pre-trial monoculture methods used on SCG-derived oligosaccharides have proved that they favour the proliferation of beneficial taxa and the production of short-chain fatty acids, suggesting true prebiotic potential; however, translating monoculture growth

Table 4
In vitro assessment used in the of prebiotic efficacy with SCG-derived MOS.

Model	Condition of assessment				Analysis used	Reference
	Time	Temperature (°C)	pH	Enzyme used		
In vitro gastrointestinal digestion	Oral phase: 5min Stomach: 2 h Small intestinal:2 h	37	Oral and small intestinal: pH=7 Stomach: pH=2	α-Amylase	Post digestion end-products was quantified by DNS	Puengsawad et al. (2021)
	4 h total	37	pH=1.5	α-Amylase trypsin	Post digestion end-products was quantified by DNS	Magengelele et al. (2023)
In vitro digestion-fermentation	Oral: 4 h Gastric: 4 h Intestinal: 4 h Faecal fermentation: 4,8,15 and 24 h	Digestion: 37 Faecal fermentation: (uncontrolled)	Faecal fermentation: pH=7.2	Human salivary α-amylase; porcine pancreatic enzymes; rat intestinal mucous enzymes	Post digestion end-products was quantified by HPLC Post fermentation end-products (SCFAs) quantified by HPLC	Asano et al. (2003)
In vitro faecal batch culture fermentation	Faecal fermentation: 24 h	Faecal fermentation: 37	Faecal fermentation: pH=7	–	SCFAs were quantified by HPLC DNA extraction and sequencing were used to quantify faecal microbial community	Perez-Burillo et al. (2019)

results into community-level effects requires such complex models to confirm efficacy *in situ*. Notably, Puengsawad et al. (2021) and Magengelele et al. (2023) also incorporated *in vitro* digestion models, but both studies relied solely on these models to determine prebiotic potential, without additional verification through more advanced analytical methods.

Asano et al. (2003) advanced this by employing an *in vitro* digestion-fermentation model, demonstrating that SCG-derived MOS was resistant to digestive enzymes in the oral, gastric, and intestinal phases. Their fermentation study showed that SCG-derived MOS produced 348.7 ± 69.9 mg of acetate after 15 h, compared to 339.8 ± 54.1 mg from fructooligosaccharides (FOS) after 8 h. This suggested that SCG-derived MOS could potentially alter the intestinal environment, though further *in vivo* research is needed to validate these findings.

A more recent study by Perez-Burillo et al. (2019) utilized an *in vitro* batch fermentation model alongside DNA sequencing and bioinformatics analysis to investigate microbial community changes in response to MOS. The study found that MOS promoted the growth of beneficial bacteria, with nearly 100 % of the bacteria belonging to the phyla Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria. Bacteroidetes, recognized for their role in producing acetate and propionate, were most abundant (>50 %) when the lowest amount of MOS was present. Proteobacteria, which play a role in metabolism and inflammation regulation, were more abundant (35 %) when the lowest MOS concentration was produced, compared to <25 % in other conditions.

In terms of total SCFAs production, higher concentrations of MOS led to greater SCFAs production. The highest SCFAs production was recorded at 251.15 ± 20.67 μmol/g of SCG, with acetate accounting for 130.79 μmol/g, propionate at 63.10 μmol/g, and butyrate at 49.07 μmol/g. This demonstrates the potential for SCG-derived MOS to positively modulate gut microbiota and enhance SCFA production.

In vivo studies are critical for validating prebiotic potential, as they allow researchers to directly observe the effects of MOS on gut health and related biomarkers (Table 5).

Asano et al. (2004) conducted one such study by recruiting eight healthy volunteers to consume 1 g/day and 3 g/day of SCG-derived MOS for eight weeks. The results indicated a significant increase in *Bifidobacterium* populations (from 9.1 % to 35.1 % of the total bacterial count) at both doses, alongside a reduction in harmful bacteria like *Clostridium perfringens*, likely due to a decrease in intestinal pH. While both doses produced significant changes, no dose-dependent effect was observed.

Table 5
In vivo assessment used in the of prebiotic efficacy with SCG-derived MOS.

Models	Human/animal study design	Analysis used	Reference
In vivo animal trials	A 12-week study, with 22 3-week-old mice being fed with high-fat diet and MOS	Not applied	Takao et al. (2006)
	A 10-week study, with 10 5-week-old Dahl-s and 5 Dahl-R (salt-resistant) rats fed with MOS	Not applied	Hoshino-Takao et al. (2008)
In vivo human clinical trials	An 8-week study, with 2 men and 6 women (18–45 years old.) Each volunteers consumed 3 g MOS per day for two weeks from week 2 to week 4 (week 0 - week 2 is the observation period), week 4 – week 6 is the interval week with no dose, week 6 – week 8 volunteers took 1g MOS per day	Traditional culture media method was used to quantify the faecal flora SCFAs were determined as fermentation products using HPLC	Asano et al. (2004)
	A double-blind, randomised, placebo-controlled 12-week study, with 54 overweight participants (19–65 years old) The intake schedule: MOS were dissolved in beverages. Each participants consumed MOS beverages twice a day (2 g of MOS contained in each beverage) for 12 weeks.	Not applied	Salinardi et al. (2010)

This study also reported an increase in total SCFAs, with acetate rising from 3.12 ± 0.33 mg/g to 3.63 ± 0.27 mg/g of faeces after consuming 3 g/day of MOS.

Takao et al. (2006) further explored the potential health benefits of SCG-derived MOS in mice fed a high-fat diet, a common model for studying metabolic disorders. Mice fed 1 % (w/w) MOS for twelve weeks exhibited lower fat accumulation and hepatic triglyceride levels, with increased faecal fat excretion compared to the control group. While this suggests that MOS may inhibit the absorption of dietary fat and prevent fat storage, the study did not explore the prebiotic potential of MOS in detail. Hoshino-Takao et al. (2008) expanded on this by studying the effects of SCG-derived MOS on blood pressure in Dahl salt-sensitive rats. After ten weeks of treatment, the rats showed significantly lower blood pressure and serum aldosterone levels, suggesting that MOS may have anti-hypertensive properties. This study highlights the broader potential health benefits of MOS beyond gut microbiota modulation. Salinardi et al. (2010) conducted a human clinical study on SCG- and coffee ground-derived MOS, focusing on weight management. In a double-blind, randomized, placebo-controlled trial with 54 participants, male subjects who consumed MOS for 12 weeks experienced significant reductions in body fat and total body volume compared to the placebo group. However, no significant effects were observed in female participants, suggesting potential gender differences in response to MOS. Further research is needed to explore the mechanisms behind these gender-specific effects.

6.3. Digestibility and fermentation of SCG solids

SCG itself has also been evaluated for its prebiotic potential. Jiménez-Zamora et al. (2015) investigated various coffee by-products, including SCG, using an *in vitro* digestion-fermentation model. Although the term "prebiotic activity" was incorrectly termed in the study, the results demonstrated that SCG could promote the growth of lactobacilli and bifidobacteria after 24 h of fermentation. However, the study did not measure the production of metabolites, which limits its ability to confirm the prebiotic potential of SCG.

López-Barrera et al. (2016) evaluated the fermentability of dark- and medium-roasted SCG using an *in vitro* digestion-fermentation model. Both types significantly increased SCFA production, with acetate, propionate, and butyrate levels at 37.5 ± 7.4 mmol/L, 9.50 ± 0.6 mmol/L, and 7.2 ± 0.3 mmol/L, respectively, though lower than MOS. The study introduced a rat-everted gut sac model to better mimic brush border enzymes and selective absorption (Alam et al., 2012). SCG did not survive the oral, gastric, or intestinal phases, suggesting it might reach the gut intact. Given the physiological differences between rats and humans, these results may not directly translate to human responses, and this model, while more complex, still cannot fully replicate the human digestive system.

Besides prebiotic potential, SCG's anti-inflammatory effects were evaluated using inflammation markers in macrophages. The results showed a 55 % reduction in nitric oxide (NO) production, suggesting that SCG may protect against chronic inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis. Although promising, *in vivo* studies are necessary to confirm these anti-inflammatory effects and their relevance to human health.

Panzella et al. (2017) further studied hydrolysed SCG using an *in vitro* digestion-fermentation model and found that hydrolysed SCG increased *Bifidobacterium* and *Lactobacillus* populations after 20 h of fermentation. SCFA production was also significantly higher compared to inulin, particularly for acetate and propionate. In addition, the study evaluated the antioxidant properties of SCG using a HepG2 cell line, revealing that hydrolysed SCG could affect cellular pathways and gene expression related to antioxidant activity. Although this study did not directly assess prebiotic potential, the antioxidant effects of SCG provide further evidence of its potential health benefits.

de Cosío-Barrón et al. (2020) evaluated medium- and dark-roasted SCG by using the same *in vitro* digestion-fermentation model as López-Barrera et al. (2016). Two types of human gut microbiota communities were used for fermentation: one from lean individuals and one from overweight individuals. Medium-roasted SCG showed better regulation of faecal enzyme activity and higher SCFA production than dark-roasted SCG. Acetate levels were highest in lean-microbiota communities, while butyrate was most abundant in overweight-microbiota groups, suggesting the microbiota composition influences SCG's prebiotic effects.

Results indicated that medium-roasted SCG is better in regulating the activity of faecal enzymes than dark-roasted; although the gut microbiota was not quantitatively profiled, medium-roasted SCG produce significantly more total short-chain fatty acids, with acetate highest in the lean-microbiota group (nearly 10 nmol/L) while butyrate was highest in the overweight-microbiota group (26.7 nmol/L), after 24 h fermentation.

Hydrolysed SCG also led to higher acetic and propionic acid production compared to probiotic milk beverages, which increased butyric acid, reflecting the role of *Bifidobacterium* spp. and *Lactobacillus* spp. in butyrate production.

In general, the conclusions drawn support the use of SCG as a functional food ingredient with potential health benefits. However, there are still questions about toxicity, due to the presence of potentially harmful compounds from the coffee beans themselves, such as caffeine, tannins, and certain polyphenols, which could pose health risks in high concentrations. Due to its complex compositional profile, it is hard to elucidate the role of its various components, such as oligosaccharides, polysaccharides and coffee polyphenols. Therefore, to better understand these functional compounds in SCG, studies on extracted single components are needed. This approach will help in identifying their specific health benefits and potential uses as functional food ingredients.

Despite the obvious potential of SCG-derived carbohydrates to act as prebiotics, none have yet accumulated sufficient evidence of prebiotic status. There is clearly a need for in *vivo* human studies, ideally comparing food-grade SCG-derived carbohydrates and SCG, with commercial oligofructose and galactooligosaccharides, in specific health conditions. In summary, well-designed human studies are essential for confirming prebiotic status and understanding their potential health benefits. These studies are expensive and time-consuming. They also require large quantities of food-grade materials, and enough participants to achieve statistical power (Spacova et al., 2020). Furthermore, these studies must show specific and quantifiable health benefits, as changes in microbiome composition alone are not adequate. Consequently, building a solid portfolio of supporting evidence through *in vivo* human studies requires long-term commitment and investment.

7. Conclusion

Numerous studies have indicated the valorisation pathway of SCG into functional food ingredients and various extraction methods have been applied. MOS are one of the most extracted functional oligosaccharides, using microbial β-mannanases but there is a challenge to produce them on a large scale. Pre-treatment methods, specific enzyme doses, and optimization of the production conditions are needed to increase the production effectiveness.

Problems exist with the evaluation of SCG-derived products; studies generally make efforts to investigate their prebiotic potential and indicate that they are all beneficial in modulating gut microbiota and metabolic products. However, very few tests have adequately proven the prebiotic status of SCG-derived compounds.

Further research should focus on the application of SCG-derived oligosaccharides at a commercial food-grade scale as novel functional food ingredient. In addition, the prebiotic status of SCG-derived compounds, especially oligosaccharides, needs to be confirmed, using well-controlled human studies. In addition to MOS, other oligosaccharides, such as cello-oligosaccharides (COS), and galactooligosaccharides

(GOS), could also be explored as possible prebiotic candidates.

CRediT authorship contribution statement

Manxi Huang: Writing – original draft, Investigation, Data curation. **Afroditi Chatzifragkou:** Writing – review & editing, Supervision, Conceptualization. **Robert A. Rastall:** Writing – review & editing, Supervision, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT to improve the readability and correct the Grammer mistakes (proof-reading). After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Data availability

Data will be made available on request.

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