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RESEARCH PAPER

Potential prebiotic effect of hydrated basil seeds (*Ocimum basilicum* L.) and hydrated chia seeds (*Salvia hispanica* L.) after *in vitro* gastrointestinal digestion

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Abstract

Basil seeds and chia seeds are commonly consumed as drink and food. Basil and chia are part of the Lamiaceae family. They have a hard inner part and an outer layer with pectin fibrils. When the outer pericarp of the seed is soaked in water, it will swell until it forms gum. Both basil and chia seeds are similar in physical characteristics – they are black, oval, and nearly the same size. Hydrated basil seeds (HBS) and hydrated chia seeds (HCS) have rarely been studied for their prebiotic potential *in vitro*. This study aimed to determine the prebiotic potential of both hydrated basil seeds and hydrated chia seeds, based on their chemical characteristics, carbohydrate resistance during simulated gastrointestinal (GI) digestion, prebiotic index and prebiotic activity using *Lactobacillus paracasei* (probiotic) and *Escherichia coli* (pathogenic). The initial step of this study was the chemical characterization of both HBS and HCS. The second step was to evaluate the carbohydrate resistance of both HBS and HCS in simulated gastrointestinal digestion by analyzing the reducing sugar content and the total carbohydrate content after GI digestion. The third step was determining the prebiotic index and prebiotic activity by testing the growth of colonic bacteria (*L. paracasei* and *E. coli*) to which HBS and HCS were added at 2.5, 5, and 10% (v/v). The results showed that lipid and protein content in HBS and HCS was quite high, with HCS having higher levels than HBS. Both HBS and HCS contained carbohydrates that resist hydrolysis by human digestive enzymes, with HCS containing a higher percentage of hydrolyzed carbohydrates (22%) compared to HBS (11%). Furthermore, HCS was found to stimulate the growth of *L. paracasei* to a greater extent than HBS. HCS 2.5% demonstrated the highest prebiotic index, even higher than inulin 2.5% as a positive control. HCS was more discerning in promoting the growth of colon bacteria compared to HBS. Based on prebiotic criteria, this study found that hydrated chia seeds (HCS) were more effective as prebiotic than hydrated basil seeds (HBS).

Keywords

Basil seed, chia seed, *E. coli*, *L. paracasei*, *Ocimum basilicum*, prebiotics, *Salvia hispanica*

Introduction

According to the International Scientific Association of Probiotics and Prebiotics (ISAPP), prebiotics are defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al. 2017). Prebiotic criteria are as follows: (i) resistant to stomach acidity, cannot be hydrolyzed by mammalian digestive enzymes, should not be absorbed by the upper gastrointestinal tract, (ii) can be fermented by intestinal microbiota, and (iii) can stimulate selective growth and activity of colon probiotics to improve host’s health (Davani-Davari et al. 2019). The most common source of prebiotics comes from resistant carbohydrates. Prebiotics are often associated with the prevention of digestive system diseases such as colon cancer, exerting positive effects on lipid metabolism, weight management, and mineral adsorption (Pokusaeva et al. 2011).

Basil (*Ocimum basilicum* L.) and chia (*Salvia hispanica* L.) are both of the Lamiceae family. Basil and chia seeds share similar physical characteristics and are commonly consumed in drinks and food. The seeds are generally black, oval, and nearly the same size (length 2.11–2.52 mm, wide 1.32–1.43 mm, and thickness 0.81–1.10). These two seeds consist of a hard inner part and an outer layer containing fibrous pectin. The outer pericarp (or epidermis) of the seeds swells and forms gum when soaked in water (Hrnčič et al. 2020; Nazir and Wani 2021). Chia and basil are usually used as food in form of HCS dan HBS. Prebiotic potential of seeds has been previously demonstrated in studies, such as mango seed, tamarind seed, buckwheat, sesame seed, and cereals (Bamigbade et al. 2022).

The prebiotic potential of hydrated chia seeds (HCS) and hydrated basil seeds (HBS) has been under-studied *in vitro*. Studies on the prebiotic potential of chia seeds and basil seeds have been conducted using their polysaccharide extracts (Wongputtisin and Khanongnuch 2015; Mihai et al. 2023). However, there are currently no reports on the prebiotic potential of hydrated chia seeds (HCS) and hydrated basil seeds (HBS). This study aimed to determine the prebiotic potential of hydrated basil seeds and chia seeds, based on the chemical characteristics of hydrated chia seeds (HCS) and hydrated basil seeds (HBS), the carbohydrate resistance of HCS and HBS in the simulated GI digestion, as well as the prebiotic index and prebiotic activity.

Materials and methods

Chia seeds (Sain Organik brand) and basil seeds (Koepoe-Koepoe brand) were purchased online. Analytical grade reagents, including hydrochloric acid (HCl), sodium oxide (NaOH), ethanol, sodium bicarbonate ($NaHCO_3$), and glucose were purchased from Merck. Pepsin enzyme (P7000), pancreatin (P1745), and bile extract (B8631) were purchased from Sigma Aldrich. Tryptic soy agar (TSA), tryptic soy broth (TSB), de Man, Rogosa, and Sharpe agar (MRSA), de Man, Rogosa, and Sharpe broth (MRSB) were purchased from Oxoid. Inulin DP > 10 from Orafti®GR. *Escherichia coli* (ATCC 25922) was taken from SEAFAST Center IPB University, and *Lactobacillus paracasei* (IPBCC.b.23.1544) was taken from IPB University Culture Collection Center.

Sample preparation and proximate analysis of HCS and HBS

Basil and chia seeds were soaked in distilled water (1:30) and incubated in a water-bath shaker at 150 rpm for 2 hours at room temperature. The hydrated seeds were blended for 20 seconds so they thickened quickly. Both hydrated chia seeds (HCS) and hydrated basil seeds (HBS) were subjected to proximate analysis, namely moisture, ash, protein, fat, and carbohydrate by difference (AOAC 2012).

Determination of HCS and HBS carbohydrate resistance in gastro-intestinal digestion simulation

Simulated GI digestion refers to Liu et al. (2016). A certain amount of HCS or HBS (derived from 1 gram of chia seeds/basil seeds and 30 mL of distilled water) was added to 20 mL of distilled water and stirred until homogeneous. Diluted HCS or HBS was digested in the gastric phase, followed by the intestinal phase. The gastric phase was carried out by lowering the pH to pH 2 with HCl 6 mol/L and then by adding pepsin (50 mg), then incubated in a water bath shaker at 200 rpm, temperature 37 °C for 2 hours. The small intestine phase was carried out by first neutralizing the acidity of the sample to pH 6.5, followed by adding 2 mL of a mixture of pancreatin (0.08 g) and bile extract (0.5 g) in a 0.5 mol/L $NaHCO_3$ solution, then incubating in water-bath shaker with speed 200 rpm, temperature 37 °C for 3 hours. The samples solution were heated in boiled water for 15 minutes to inactivate the enzymes. To determine the percentage of carbohydrate hydrolysis, samples were taken from the pre-digestive phase (BD), post-gastric phase (GP), and post-intestinal phase (IP). Inulin was used as control and conducted with the same procedure as HBS and HCS.

The three-phase aliquot samples were analyzed for total carbohydrate content using the phenol-sulfuric acid method (Goh et al. 2016). Briefly, sample was dissolved in ethanol 80% until its volume reached 100 mL and then heated in water-bath at 65–70 °C for 15 minutes to extract the reducing sugar. The filtered extract was taken 1 mL and put into a test tube, and 3 mL of DNS solution was then added. The reaction tube was heated in a water bath at 100 °C for 5 minutes, then cooled immediately. The absorbance of the sample was measured using a UV-Vis spectrophotometer with a wavelength of 540 nm.

Determination of reducing sugar content was carried out using the DNS method (Glibowski and Bukowska 2011). Briefly, sample was dissolved in distilled water until its volume reached 100 mL. One mL of sample solution was put into a test tube, and 1 mL of 5% phenol solution was added and then homogenized using a vortex. Then the solution was added with 5 mL of concentrated sulfuric acid solution (95%). The solution was incubated for 10 minutes in a water bath at 30 °C. The absorbance of the solution was then measured using a UV-Vis spectrophotometer with a wavelength of 490 nm.

Assessment of the selectivity ability of colonic bacterial growth

Sample preparation

This preparation stage was carried out to remove the sugar content in post-intestinal phase of HBS, HCS, and inulin (or HBS-IP, HCS-IP, and Inulin-IP) using 80% ethanol (Biswas et al. 2022 with modification). HBS-IP, HCS-IP, and Inulin-IP were added with 80% ethanol (1:3) and heated in a water-bath at 65–70 °C for 15 minutes. The sample was centrifuged at 3000 rpm for 10 minutes at 4 °C, filtered, and the residue was taken. The residue was then rinsed with distilled water and centrifuged at 3000 rpm for 10 minutes at 4 °C, then filtered, and the residue was taken. The rinsing stage using distilled water was carried out three times to reduce the residual ethanol level.

Inoculation and counting of colonic bacterial growth

The procedure refers to Dawood et al. (2021) and Liu et al. (2016). Sugar-free HBS-IP, HCS-IP, or Inulin-IP samples were added to the minimum media (mMRSB and M9) at different concentrations (2.5%, 5%, and 10%, v/v). The broth media was then inoculated overnight with 1% (v/v) culture of *Lactobacillus paracasei* (probiotic) or *Escherichia coli* (pathogen). The colonies were counted at 0 h and 24 h of incubation at 37 °C. The number of microflora colonies was counted at 0 h, as follows. The minimum media containing the sample and bacteria was diluted to a dilution of 10^{-7}. The last three dilutions (10^{-5}, 10^{-6}, 10^{-7}) were taken as 1 mL and then inoculated into agar media using the pour method. The agar media used was MRSA for *L. paracasei* and TSA for *E. coli*. MRSA media was incubated for 48 hours at 37 °C, while TSA media was incubated for 24 hours at 35 °C. Quantification of bacterial growth in broth media after 24 h incubation was carried out in the same way as described above.

As a comparison, counting the number of bacteria was also carried out using minimal media with added glucose 2.5%. The number of colonies was then used in the formula below to calculate the prebiotic index (PI) (Dawood et al. 2021) and prebiotic activity (PA) (Huebner et al. 2007).

$$\mathrm{PI} = \frac{\text{CFU of } L.\,paracasei \text{ on sample}}{\text{CFU of } L.\,paracasei \text{ on inulin (control)}}$$

$$\mathrm{PA} = \frac{\left(\log \frac{\mathrm{CFU}}{\mathrm{g}} 24\mathrm{h} - \log \frac{\mathrm{CFU}}{\mathrm{g}} 0\mathrm{h}\right) L.\ paracasei \text{ on sample}}{\left(\log \frac{\mathrm{CFU}}{\mathrm{g}} 24\mathrm{h} - \log \frac{\mathrm{CFU}}{\mathrm{g}} 0\mathrm{h}\right) L.\ paracasei \text{ on glucose}} - \frac{\left(\log \frac{\mathrm{CFU}}{\mathrm{g}} 24\mathrm{h} - \log \frac{\mathrm{CFU}}{\mathrm{g}} 0\mathrm{h}\right) E.\ coli \text{ on sample}}{\left(\log \frac{\mathrm{CFU}}{\mathrm{g}} 24\mathrm{h} - \log \frac{\mathrm{CFU}}{\mathrm{g}} 0\mathrm{h}\right) E.\ coli \text{ on glucose}}$$

Statistical analysis

All data are presented as mean values ± standard error of the mean determined in triplicate. Proximate data were analyzed using a single sample t-test with Minitab 18. To evaluate the carbohydrate resistance in simulated digestion and the effect of carbon source on the bacterial growth, data were analyzed by one-way ANOVA followed by the Tukey HSD. Results were considered as statistically significant when the p-value was less than 0.05.

Results and discussion

The proximate composition of HCS and HBS

Fig. 1 showed the appearance of HBS and HCS. The results of proximate analysis of hydrated chia seeds (HCS) and hydrated basil seeds (HBS) are shown in Table 1. All proximate contents in HBS were significantly different ($P < 0.05$) from HCS. The fat and lipid content of HBS and HCS were relatively high, namely fat content of 34.01% and 43.94%, and protein content of 31.98% and 37.75%, respectively. The moisture, fat and protein content of HCS was significantly higher than HBS, while HBS had much higher ash and carbohydrate content. High levels of lipids and proteins affect the amount of carbohydrates that can be digested. Interactions among starch, lipids, and proteins have been found to influence starch digestibility. Additionally, these interactions may lead to the formation of resistant starch, which can be fermented by gut microbiota in the colon, providing several health benefits (Wang et al. 2024). Moreover, lipids impact the gut microbiota by serving as substrates for bacterial metabolic

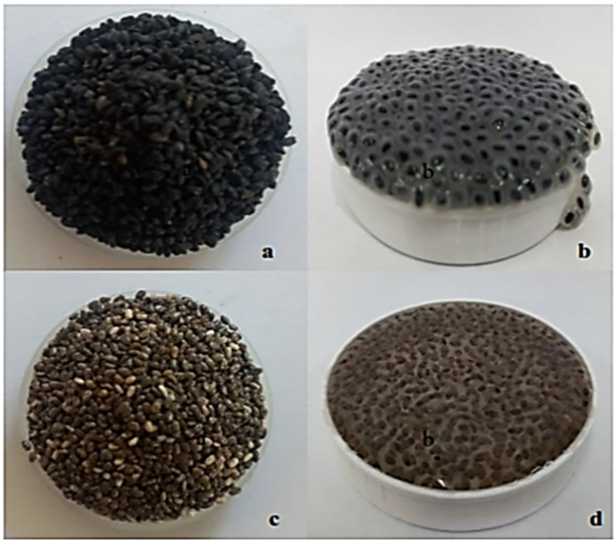

Figure 1. Basil seeds (**a**), hydrated basil seeds (HBS) (**b**), chia seeds (**c**), and hydrated chia seeds (HCS) (**d**).

Table 1. Proximate composition of hydrated basil seeds (HBS) and hydrated chia seeds (HCS).

	Hydrated basil seeds (HBS)		Hydrated chia seeds (HCS)	
	Wet basis (%)	Dry basis (%)	Wet basis (%)	Dry basis (%)
Water	95.56 ± 0.03^{b}	-	96.45 ± 0.03^{a}	-
Ash	0.33 ± 0.00	7.43 ± 0.02^{a}	0.16 ± 0.00	4.51 ± 0.03^{b}
Fat	1.51 ± 0.01	34.01 ± 0.36^{b}	1.56 ± 0.01	43.94 ± 0.79^{a}
Protein	1.42 ± 0.00	31.98 ± 0.27^{b}	1.34 ± 0.02	37.75 ± 0.18^{a}
Carbohydrate	1.18 ± 0.04	26.57 ± 0.64^{a}	0.49 ± 0.03	13.80 ± 0.64^{b}

Different letters within the same row indicate a significant difference ($P < 0.05$).

activities and by exerting toxic effects that inhibit bacterial growth (Schoeler and Caesar 2019). Protein hydrolysates have been shown to promote the expression of beneficial traits in probiotics (Gao et al. 2023). Dietary fiber in HBS and HCS were high (55.4% and 44.4%) (Rahmawati et al. 2024). The amount of undigested fraction can be an indicator of prebiotic potential. However, not all carbohydrates in this fraction can be fermented by colonic microbes.

Carbohydrate resistance of HCS and HBS in simulated gastro-intestinal digestion

Reducing sugars and total carbohydrate content of HCS and HBS are shown in Table 2. The low amount of reducing sugars in the gastric (GP) and intestinal (IP) phases indicated low levels of hydrolyzed carbohydrates in the digestive tract.

Table 3. Hydrolyzed carbohydrate percentage of hydrated basil seeds (HBS), hydrated chia seeds (HCS), and inulin, respectively in the pre-digestive phase (BD), post-gastric phase (GP) and post-intestinal phase (IP).

Sample	Hydrolyzed carbohydrate (%)[1]		
	GP	IP	Total
HBS	9.920 ± 0.46^{b}	0.938 ± 0.54^{a}	10.858 ± 0.54^{b}
HCS	20.979 ± 1.84^{a}	0.967 ± 1.22^{a}	21.946 ± 1.84^{a}
Inulin	`1.889 ± 0.22^{c}	0.626 ± 0.32^{a}	2.515 ± 0.28^{c}

[1] Hydrolyzed carbohydrate$_{GP}$ = 100*(RS$_{GP}$ – RS$_{BD}$)/(Total CHO$_{BD}$-RS$_{BD}$). Hydrolyzed carbohydrate$_{IP}$ = 100*(RS$_{IP}$ – RS$_{GP}$)/(Total CHO$_{GP}$-RS$_{GP}$). Different letters within the same column indicate a significant difference ($P < 0.05$).

Before the digestion stage (BD), there was no difference in the total reducing sugar levels of HCS and HBS, while the total carbohydrates of HBS were much higher than HCS. The reducing sugar content of HBS-BD is higher than that of dried basil seeds of the Qom variant from Iran, namely 0.12% (Naji-Tabasi and Razavi 2017). It could be that the hydration process in basil seeds causes an increase in reducing sugar levels. Reducing sugar levels experienced a significant increase from HBS-BD to HBS-GP ($P < 0.05$), while from HBS-GP to HBS-IP, there was no significant increase ($P > 0.05$). The same pattern was also found in HCS. The increase in reducing sugar levels is caused by a decrease in pH to 2, which mimics the digestive process in the stomach. Low pH can cause hydrolysis of polysaccharides and they can be broken down into shorter polysaccharides, disaccharides, oligosaccharides and monosaccharides (Lu et al. 2021). In inulin as a positive control, reducing sugars increased significantly ($P < 0.05$), both from BP to GP and GP to IP. Glibowski and Bukowska (2011) stated that a decrease in pH affects the levels of inulin reducing sugars; in a pH < 4 environment, the levels of inulin reducing sugars are higher than in a pH > 4 environment. The low amount of reducing sugars in IP indicates that most of the carbohydrates in HBS and HCS cannot be digested into simple sugars, such as glucose or maltose. Chia seeds contain reducing sugars such as mannose, xylose, glucose, galactose, and arabinose. Chia seeds also contain acidic sugars as well as glucuronic acid and galacturonic acid (Hernandez 2012; Timilsena et al. 2016). The reducing sugars found in basil seeds are glucose, galactose, mannose, arabinose, xylose, and rhamnose (Naji-Tabasi et al. 2016).

In general, total carbohydrates in HBS were higher than in HCS ($P < 0.05$). Complex carbohydrates that are resistant to gastric and intestinal digestion can be calculated by subtracting reducing sugars from total carbohydrates. Furthermore, complex carbohydrates in IP describe the portion of carbohydrates that reached the colon phase. The data showed that the complex carbohydrates of all samples experienced a slight decrease after the gastric phase, but there was no change after the intestinal phase.

The percentage of hydrolyzed carbohydrates in HBS, HCS, and inulin (as control) are shown in Table 3. In the GP sample, the percentage of hydrolyzed carbohydrates of HCS was 20.9%, which was significantly higher ($P < 0.05$) compared to 9.9% in HBS and 1.9% in inulin. The percentage of hydrolyzed carbohydrate in IP was not significantly different ($P > 0.05$) among all samples, with values consistently below 1%. The highest total hydrolyzed carbohydrate was HCS (22.0%), followed by HBS (11%), and inulin (2.5%). Based on the amount of carbohydrates complex af-

Table 2. Reducing sugar and total carbohydrate content of hydrated basil seeds (HBS), hydrated chia seeds (HCS), and inulin, respectively in the pre-digestive phase (BD), post-gastric phase (GP) and post-intestinal phase (IP).

Sample	Reducing sugar (RS) (%db)	Total carbohydrate (CHO) (%db)	Complex carbohydrate (%db)	Complex carbohydrate (% from total carbohydrate)
	(1)	(2)	(3) = (2)-(1)	(4) = 100*(3)/(2)
HBS-BD	2.774 ± 0.09^{e}	23.547 ± 0.11^{d}	20.773 ± 0.22^{d}	88.219 ± 0.52^{b}
HBS-GP	4.834 ± 0.15^{d}	27.415 ± 0.48^{c}	22.581 ± 0.65^{cd}	82.367 ± 0.96^{c}
HBS-IP	5.046 ± 0.12^{d}	29.112 ± 0.30^{c}	24.066 ± 0.44^{c}	82.667 ± 0.67^{c}
HCS-BD	2.430 ± 0.10^{e}	17.945 ± 0.78^{e}	15.515 ± 0.70^{e}	86.459 ± 0.49^{b}
HCS-GP	5.685 ± 0.02^{c}	21.505 ± 0.35^{d}	15.820 ± 0.33^{e}	73.564 ± 0.35^{d}
HCS-IP	5.838 ± 0.14^{c}	23.054 ± 0.48^{d}	17.216 ± 0.67^{e}	74.677 ± 1.37^{d}
Inulin-BD	4.894 ± 0.17^{d}	97.079 ± 0.34^{b}	92.185 ± 0.50^{b}	94.959 ± 0.19^{a}
Inulin-GP	6.636 ± 0.16^{b}	104.495 ± 1.36^{a}	97.859 ± 1.48^{a}	93.649 ± 0.22^{a}
Inulin-IP	7.250 ± 0.16^{a}	105.989 ± 1.72^{a}	98.739 ± 1.83^{a}	93.160 ± 0.24^{a}

Different letters within the same column indicate a significant difference ($P < 0.05$).

ter digestion and the percentage of carbohydrates hydrolyzed, it indicates that HBS has a higher undigested carbohydrate content than HCS, but both are still lower than inulin. Despite the high levels of undigested carbohydrates, not all of them can be fermented by colonic microbes.

Effect of HCS and HBS on the growth of *L. paracasei* and *E. coli*

The increase in bacterial growth in HCS, HBS, glucose, and inulin (control) is presented in Table 4. Inulin was used as a comparison because it is a prebiotic, which is widely used as a food mixture. Inulin (purity 99%, degree of polymerization 2–60) was able to grow *L. paracasei* bacteria with an increase of Log 2.63 (Huebner et al. 2007). Glucose was used in this study to determine the prebiotic index value and prebiotic activity. Glucose, being the simplest sugar, is readily utilized by *L. paracasei* and *E. coli*. However, glucose is not classified as a prebiotic due to its ability to be absorbed in the small intestine.

In this study, the number of *L. paracasei* and *E. coli* colonies in all samples at hour 0 was approximately 7.8 Log CFU/g and 7.3 Log CFU/g, respectively. Specifically, the number of *L. paracasei* bacteria after 24 hours on sugar-free HBS and HCS ranged from 8.0–8.7 Log CFU/g, while for *E. coli* bacteria it ranged from 7.5–8.0 Log CFU/g. The highest increase in *L. paracasei* cells occurred in HCS at 2.5% (0.88 Log) with a cell count of 8.7 Log CFU/g, which was not statistically different from inulin at 2.5% (0.93 Log). The growth of *L. paracasei* on 2.5% HCS was close to the growth of *L. casei* on basil seed oligosaccharide extract, i.e. 8.9 Log CFU/g after incubation for 24 hours (Wongputtisin and Khanongnuch 2015). The amount of *L. paracasei* with the nutritional source inulin can reach 8.9 Log CFU/g (Takagi et al. 2014). The lowest increase in *L. paracasei* cells was found in 10% HBS (0.2 Log) with a cell count of 8.0 Log CFU/g. Basil seeds showed antimicrobial activity against various Gram-negative and Gram-positive bacteria, yeast and fungi (Suppakul et al. 2003).

In samples tested with *E. coli*, the lowest increase in the number of *E. coli* was found in HCS 10% and HBS 10% by 0.2 Log to 7.5 Log CFU/g. In this study, both HCS and HBS were able to suppress the growth of *E. coli* better than inulin. Chia seeds and basil seeds have antibacterial ability due to their omega-3 fatty acid content (Bravo et al. 2021; Motyka et al. 2023).

Ethanol at 80% concentration used to remove reducing sugars generated during gastrointestinal digestion, can also dissolve oligosaccharides with a low degree of polymerization (DP) (Xu et al. 2014). As a result, the remaining compounds in HBS and HCS consist primarily of polysaccharides with a high DP. The degree of polymerization (DP) of carbohydrates significantly influences their utilization by probiotics. Carbohydrates with a DP of 3 to 4 exhibit the most effective prebiotic activity, whereas those with a DP above 5 show reduced effects (Lu et al. 2021).

In addition to polysaccharides, other components such as resistant starch, polyunsaturated fatty acids (PUFA), and phenolic compounds also demonstrate prebiotic properties. For example, resistant starch from lotus seeds promotes the growth of *Bifidobacteria*, while resistant starch from oats and sorghum supports the growth of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactobacillus delbrueckii* (Zhang et al. 2014; Chen et al. 2022).

Polyphenols and PUFA also possess prebiotic properties (Plamada and Vodnar 2022). The PUFA content in chia seeds and basil seeds are dominated by linolenic acid, followed by linoleic acid and oleic acid (Bravo et al. 2021; Motyka et al. 2022). PUFA have been shown to modulate the gut microbiota by altering the Firmicutes/Bacteroidetes (F/B) ratio and increasing the abundance of butyrate-producing bacteria, such as Bifidobacterium, Lachnospira, Roseburia, and Lactobacillus (Rehman et al. 2024).

Chia seeds are rich in polyphenolic compounds, which can be present in a free state or bound to sugars through glycosidic bonds. The polyphenols identified in chia seeds include phenolic acids (such as gallic acid, caffeic acid, ferulic acid, and p-coumaric acid), depsides (chlorogenic acid and rosmarinic acid), flavonoids (apigenin, kaempferol, quercetin, myricetin, and rutoside), isoflavones (including daidzein, glycitin, genistein, and genistin), as well as catechin derivatives like epicatechin (Motyka et al. 2022). Orientine, vicentine, and rosmarinic acid are phenolic

Table 4. The growth of *L. paracasei* and *E. coli* (Log CFU/g) on media supplemented with post-intestinal phase of hydrated basil seeds (HBS), hydrated chia seeds (HCS), or inulin.

Sample	*L. paracasei* (Log CFU/g)			*E. coli* (Log CFU/ g)		
	0h	24h	Δ	0h	24h	Δ
Wihout sample	7.73 ± 0.02^{a}	8.08 ± 0.01^{e}	0.35 ± 0.04^{e}	7.51 ± 0.06^{a}	7.92 ± 0.01^{c}	0.41 ± 0.06^{d}
Glucose 2.5%	7.82 ± 0.03^{a}	9.74 ± 0.04^{a}	1.92 ± 0.05^{a}	7.13 ± 0.09^{c}	9.07 ± 0.01^{a}	1.94 ± 0.08^{a}
Inulin 2.5%	7.43 ± 0.59^{a}	8.36 ± 0.08^{d}	0.93 ± 0.06^{b}	7.26 ± 0.02^{bc}	8.17 ± 0.04^{b}	0.91 ± 0.02^{b}
HCS 2.5%	7.81 ± 0.02^{a}	8.69 ± 0.04^{b}	0.88 ± 0.05^{b}	7.37 ± 0.06^{ab}	7.97 ± 0.01^{c}	0.60 ± 0.07^{c}
HCS 5%	7.79 ± 0.02^{a}	8.52 ± 0.01^{c}	0.73 ± 0.02^{c}	7.31 ± 0.04^{b}	7.70 ± 0.01^{e}	0.39 ± 0.05^{d}
HCS 10%	7.80 ± 0.03^{a}	8.44 ± 0.01^{cd}	0.64 ± 0.03^{d}	7.30 ± 0.06^{b}	7.51 ± 0.02^{g}	0.21 ± 0.08^{e}
HBS 2.5%	7.82 ± 0.02^{a}	8.09 ± 0.06^{e}	0.27 ± 0.04^{ef}	7.36 ± 0.06^{ab}	7.97 ± 0.06^{c}	0.61 ± 0.07^{c}
HBS 5%	7.81 ± 0.03^{a}	8.07 ± 0.01^{e}	0.26 ± 0.02^{f}	7.27 ± 0.03^{bc}	7.78 ± 0.03^{d}	0.51 ± 0.08^{cd}
HBS 10%	7.79 ± 0.01^{a}	7.99 ± 0.00^{e}	0.20 ± 0.02^{g}	7.30 ± 0.03^{b}	7.59 ± 0.03^{f}	0.29 ± 0.03^{e}

Different letters within the same column indicate a significant difference ($P < 0.05$).

compounds in basil seeds (Bravo et al. 2021). The prebiotic index shows the ability of a prebiotic candidate to stimulate the growth of probiotic bacteria (*L. paracasei*) compared to the prebiotic control (inulin 2.5%). Meanwhile, prebiotic activity indicates the level of selectivity of a prebiotic candidate in stimulating probiotic bacteria (*L. paracasei*), while suppressing pathogenic bacteria (*E. coli*). The prebiotic index and prebiotic activity in hydrated basil seeds and hydrated chia seeds are shown in Fig. 2. The prebiotic index of HCS 2.5% was 2.16, meanwhile, HCS 10% showed the highest prebiotic activity score (0.22). The HBS prebiotic index only ranged from 0.43 to 0.55, indicating that HBS did not stimulate the growth of *L. paracasei* compared with 2.5% inulin. The prebiotic index and prebiotic activity of a test sample are influenced by the composition of undigested carbohydrates, the probiotic bacteria used for testing, and the presence of antibacterial compounds in the test sample. It's important to note that this study only explored prebiotic activity using *L. paracasei*, and further research involving other probiotics is needed.

Dawood et al. (2021) reported the prebiotic index (PI) and prebiotic activity (PA) scores of Cassia fistula polysaccharide (CFP) using 4 probiotic strains. *L. casei* grew faster at high CFP concentration (2%) compared to low CFP concentrations (1.5 and 1%). The PI and PA scores of different CFP concentrations produced by *L. casei* were higher than those produced by *L. rhamnosus*. The highest values for prebiotic index (1.25) and prebiotic activity (1.9) score were observed in *L. casei* treated with 2% CFP.

Long-term consumption of chia seeds has been associated with various health benefits. In their review, Motyka et al. (2022) highlighted a study involving 25 patients with non-alcoholic fatty liver disease (NAFLD) and insulin resistance, where dietary supplementation with 25 g/day of powdered chia seeds for eight weeks led to reductions in body weight, waist circumference, total cholesterol, and triglyceride levels (from 1.9 to 1.6 mmol/L) in the intervention group. Additionally, a clinical trial on patients with type 2 diabetes demonstrated that consuming 15 g of chia seeds per 1000 kcal daily for 12 weeks significantly reduced high-sensitivity C-reactive protein (CRP) levels by 40% and von Willebrand factor levels, a key clotting component, by 21%. Basil seeds have a long history of use in Chinese and Ayurvedic medicine. Basil seeds are an excellent source of minerals, high in fiber (including pectin), and rich in flavonoids and other polyphenols (Shahrajabian and Sun 2023). However, there have been no reports of clinical studies on the effects of long-term basil seed consumption, so further research is needed.

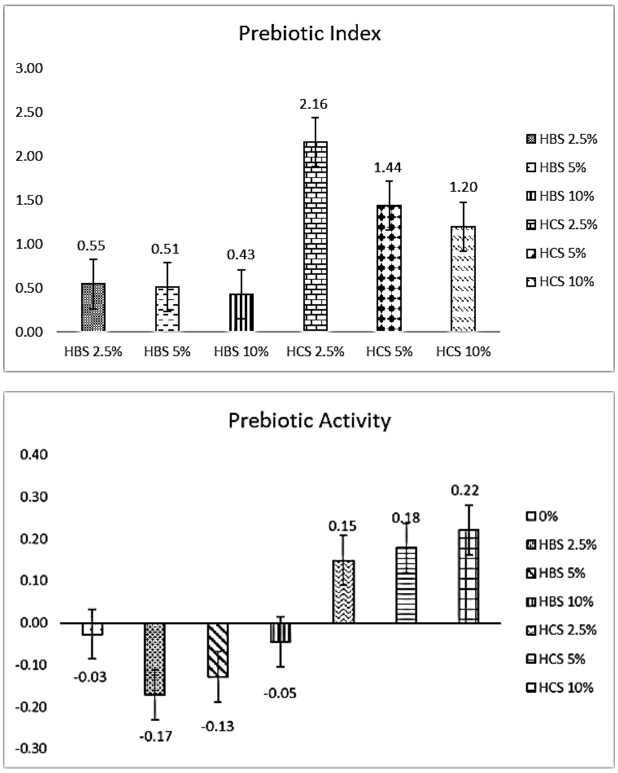

Figure 2. Prebiotic index and prebiotic activity of HBS and HCS.

Conclusions

The hydrated basil seeds (HBS) exhibit greater resistance to carbohydrate hydrolysis during gastrointestinal digestion compared to the hydrated chia seeds (HCS). However, the HCS promotes the growth of *L. paracasei* to a far greater extent than HBS. Furthermore, HCS proves to be more selective in fostering the growth of beneficial intestinal bacteria, stimulating the growth of *L. paracasei* and suppressing *E. coli*, as opposed to HBS. The hydrated chia seeds (HCS) were more effective as prebiotics than hydrated basil seeds (HBS). Further research is needed to study the prebiotic potential of hydrated chia seed and hydrated basil seed, such as identifying types of carbohydrate in HBS and HCS, short-chain fatty acids (SCFAs) produced after fermentation, conducting prebiotic assay with other probiotic bacteria, and in vivo assay.

Author's contributions

Endang Prangdimurti: supervision, interpretation of results, writing original draft, review and editing manuscript. Medina Alia Rahmawati: execution of experimental work, methodology, writing original draft. Dede Robiatul Adawiyah: supervision, designed the research plan, funding the research, resources, research project leader.

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