ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Nutrition in Health and Disease

Gastrointestinal Tolerance and Microbiome Response to Snacks Fortified with Pea Hull Fiber: A Randomized Trial in Older Adults

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ABSTRACT

Background: Consuming foods with added fiber may help older adults achieve fiber recommendations; however, many high-fiber ingredients have little effect on laxation and may contribute to unpleasant gastrointestinal side effects.

Objectives: The aim of the study was to determine the effects of consuming snacks fortified with pea hull fiber (PHF) on stool frequency and form, gastrointestinal symptoms, and appetite in older adults. An exploratory aim was to determine if PHF altered the microbiota profile.

Methods: A 10-wk, randomized, blinded, crossover study was carried out. Following a 2-wk baseline period, participants [aged (mean ± SD) 69.7 ± 6.5 y; $n = 31$; 14 men, 17 women] consumed snacks providing 10 g/d of PHF or a control, each for 2-wk periods followed by 2-wk washouts. Participants used the Bristol Stool Form Scale (BSFS) to record daily stool frequency and gastrointestinal symptoms, and completed the Gastrointestinal Symptom Rating Scale (GSRS) and Simplified Nutritional Appetite Questionnaire (SNAQ) biweekly. One stool was collected per period for 16S ribosomal RNA high-throughput amplicon sequencing of the fecal microbiota profile.

Results: Participants reported 1.63 ± 0.05 stools/d and 76.6% normal transit stool form at baseline and no change with PHF. GSRS syndrome scores were similarly unchanged. Daily abdominal noises and bloating were higher for PHF versus control, and flatulence was higher for PHF versus baseline, suggesting fermentation in some individuals. There was no evidence to suggest a common PHF-induced microbiome response for the group as a whole; however, a subgroup of participants ($n = 7$) who responded with increased flatulence (fermenters), harbored many different taxa than nonfermenters, and demonstrated lower abundance of Clostridiales with PHF. Appetite was unchanged with PHF.

Conclusions: PHF did not modulate stool form or frequency in older adults with normal bowel habits. Because snacks fortified with PHF did not suppress appetite, PHF may be an appropriate fiber source for older adults at nutritional risk. Microbiome profile may be predictive of gastrointestinal symptom response to PHF. This trial was registered at www.clinicaltrials.gov as NCT02778230. *Curr Dev Nutr* 2020;4:nzaa005.

Keywords: dietary fiber, pea hull, microbiota, gastrointestinal tolerance, sensory evaluation, GSRS, SNAQ, appetite, fermentation, older adults

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Supplemental Tables 1 and 2 and Supplemental Figures 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: BSFS, Bristol Stool Form Scale; GSRS, Gastrointestinal Symptom Response Scale; LDA, linear discriminant analysis; LefSe, linear discriminant analysis effect size; PHF, pea hull fiber; SNAQ, Simplified Nutritional Appetite Questionnaire.

Introduction

Consuming foods with added fiber may help to achieve fiber recommendations (1). This may be particularly important for older adults who may require higher-protein diets to preserve muscle mass or for restoration in times of recovery from unintentional weight loss, illness, or injury (2). Increased intakes of protein from animal-based foods, devoid of naturally occurring fiber, may displace plant-based foods containing fiber, such as whole grains, fruits, and vegetables, and thus may further support the need for fiber fortification. However, fiber ingredients, such as fructans (e.g., fructo-oligosaccharides and inulin), commonly added to foods in North America have been shown to contribute to gastrointestinal complaints including flatulence, bloating, and abdominal pain (3, 4), symptoms that may negatively impact quality of life in older adults. In addition, there is some evidence that higher intakes of fructans may suppress appetite and decrease body weight (5, 6). As appetite suppression and its possible effects on food intake and body weight may be contraindicated in older adults at nutritional risk, exploration of the physiological effects of alternative fibers for the purpose of fortification is needed.

Another consideration when choosing a fiber ingredient for fortification of food intended for older adults is its potential impact on the gut microbiota and associated health effects. Prebiotics such as fructans, given their specific carbohydrate structure, require unique enzymes for hydrolysis and thus, by definition, have specific effects on the gut microbiota or its activity (7). Although these effects, such as the enhancement of *Bifidobacterium*, are considered positive, isolated fructans have not been shown to impact diversity of the microbiota (8). This is an important consideration, as microbiota diversity is associated with stability—protection from disruption by dietary changes (9) and other stressors (10). As decreased diversity is associated with the onset of frailty (11) and increased risk of *Clostridium difficile* diarrhea (12), a potentially fatal disease more common in older adults (13), maintaining microbiota diversity is particularly important for older adults. Diets high in complex plant fibers such as whole grains are associated with higher microbial diversity (14, 15). Added fibers such as brans, containing a variety of indigestible polysaccharides, also may support microbial diversity (16). It is not known if hull fibers (pulse seed coats) have the potential for such effects. Of note, the nondigestible polysaccharides contained in the complex dietary fiber fractions of brans and hulls may be more slowly fermented than purified, soluble, and highly fermentable oligosaccharides and, thus, may produce less noticeable bloating and flatulence and, perhaps, improved acceptance and feasibility while still inducing beneficial shifts in the microbiota.

Pea hull fiber (PHF), a naturally occurring dietary fiber produced from grinding of the outer hulls of yellow field peas (17), has been shown to benefit laxation in older adults reporting low stool frequency (18). Given its complex dietary fiber constituents (i.e., cellulose, pectin, and hemicelluloses), it potentially may enhance gut microbial diversity and profile. As slow transit, suggestive of constipation, negatively impacts the gut microbiota and its metabolism (19), the potential of PHF to modulate stool form, a proxy for transit time, and stool frequency is also of interest. In addition, given that some older adults may be at risk of weight loss and malnutrition, the impact of PHF fortification on appetite requires exploration. The aims of the study were to determine the effects of daily consumption of snacks fortified with PHF on stool form, stool frequency, gastrointestinal symptoms, and appetite in community-dwelling older adults. As appetite may be impacted by the sensory acceptability of the food vehicle used for fiber fortification, sensory evaluation of the study snacks was carried out prior to the trial. In addition, an exploratory aim was to assess the effect of PHF on the microbiota profile.

Methods

Product development and sensory evaluation

Snacks (cinnamon mixed berry and oatmeal raisin chocolate chip cookies) containing 5 g of PHF (Best Cooking Pulses) and control snacks with no added fiber were developed and evaluated for acceptability in 2 sensory panels, older adults and all ages. Commercial higher fiber cookies (Belvita® Mixed Berry and FiberOne® Sugar Cookie, FiberOne® Oatmeal Raisin Cookie and Belvita® Oats & Chocolate) were used as benchmarks. Panelists (aged ≥60 y and all ages) rated samples using a hedonic 9-point scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) with questions regarding the liking of sweetness, texture, flavor, moistness, and overall acceptability of the snacks. Sensory evaluation was approved by the University of Florida Institutional Review Board 2.

Intervention study design

A 10-wk randomized, double-blind, crossover study was carried out in Florida (**Figure 1**) in 2 cohorts, July to October 2016 and May to July 2017. Following a 2-wk baseline period, participants consumed 2 cookies/d providing 10 g of PHF (9.3 g/d of fiber) or no added fiber (control) each for 2-wk periods separated by 2-wk washouts. The randomization was conducted by an individual unaffiliated with the study using a sealed, stacked-envelope method. All study participants, investigators, staff, and the statistician were blinded to the sequence until the statistical analyses were completed. Although study foods were visually very similar, there was the possibility that participants could detect sensory differences between the control and PHF fiber cookies. To determine if blinding was successful, participants were asked at the end of each intervention period, which treatment, fiber or no fiber, they thought they had been consuming. Participants recorded compliance to study food intake in the daily questionnaire and returned any uneaten study foods at each study visit.

Gastrointestinal symptoms, stool frequency and form, appetite, dietary intake, and compliance were assessed throughout the trial and stool samples were collected for sequencing. The trial was approved by the University of Florida Institutional Review Board 1. The protocol is reported on clinicaltrials.gov (registration no. NCT02778230). All participants provided written informed consent. The study was carried out in accordance with the Declaration of Helsinki.

Participants

Participants (aged ≥60 y) were recruited from the community through posters, flyers, announcements, and community and newspaper advertisements. Participants were included if they were willing to undertake study procedures and excluded if they had any known food allergies, were taking medications for diarrhea, had taken antibiotics within the past 4 wk prior to randomization, were taking probiotics supplements and did not want to discontinue a minimum of 2 wk prior to the study, or had previously been or were being treated for any diseases or illnesses such as gastrointestinal disease (gastric ulcers, Crohn's, celiac, ulcerative colitis, etc.).

Outcome measures

The primary outcomes of interest were stool form and frequency. Secondary outcomes were gastrointestinal symptoms and appetite. Participants recorded stool frequency and form using the Bristol Stool Form Scale (BSFS) (20) in daily questionnaires. Stools were categorized into slow transit (types 1 and 2), normal transit (types 3–5), and fast transit (types 6 and 7). In the daily questionnaire, participants were asked to rank daily abdominal cramping, abdominal noises, bloating, constipation, diarrhea, and flatulence using a scale from 0 (not at all) to 6 (very severe) and appetite using a scale from 0 (very poor) to 6 (very good) in response to the question, "In general, how was your appetite today?" Any changes in medications, supplement intake, and physical activity also were recorded. The Gastrointestinal Symptom Rating Scale (GSRS), a 7-point Likert scale, where 1 represents no discomfort at all and 7 represents very severe discomfort (21, 22), was administered

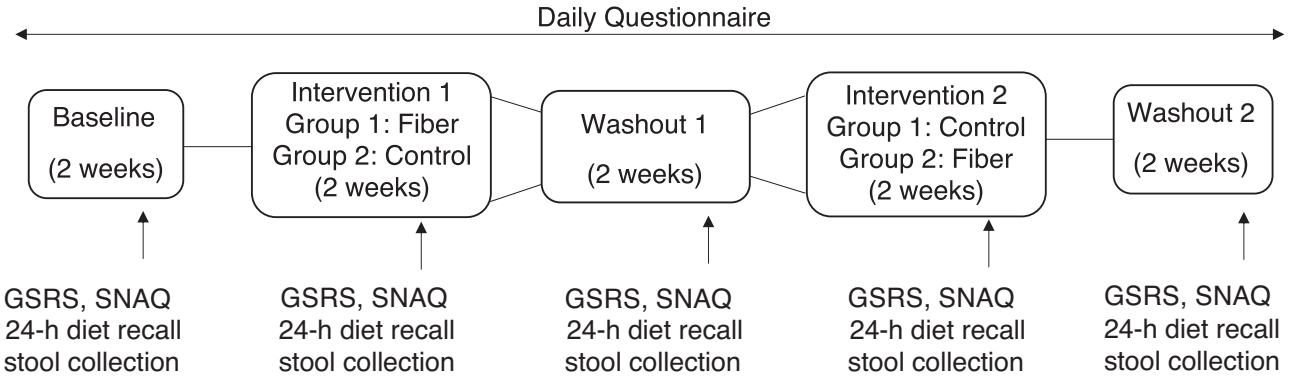

FIGURE 1 Study design of the intervention trial in older adults examining the effects of pea hull fiber. GSRS, Gastrointestinal Symptom Response Scale; SNAQ, Simplified Nutritional Appetite Questionnaire.

biweekly. Symptoms of the GSRS were combined into the 5 syndromes: reflux, abdominal pain, indigestion, diarrhea, and constipation. The Simplified Nutritional Appetite Questionnaire (SNAQ) (23) also was administered biweekly. Total scores range from 4 to 20 with a total SNAQ score ≤14 indicating poor appetite and a significant risk of weight loss for community-dwelling older adults (23). Dietary intake (24-h recall) was assessed by phone interview during each study period. Food Processor Nutrition Analysis Software (ESHA version 11.3.2) was used to analyze dietary intake.

Microbiota analysis

An exploratory outcome was microbiome profile. One stool was collected toward the end of each 2-wk period. Stools were collected using the Fisher Scientific Commode Collection System (Fisher Scientific catalog no. 02-544-208), kept on ice, and processed within 6 h of defecation. Samples were homogenized, placed in aliquots, and stored at −80°C. Total DNA was extracted from homogenized feces using the QIAamp® Fast DNA Stool Mini Kit (Qiagen) as per the manufacturer's instructions with modifications as previously described (24). Libraries for sequencing were prepared according to Illumina's 16S Metagenomic Sequencing Library Preparation guidelines (Part no. 15,044,223 Rev. B), with exceptions as previously reported (24). Template-specific primers targeted the V3–V4 region of the 16S ribosomal RNA gene (PMCID: PMC3592464) (25). Resulting sequence reads were analyzed as previously described (26, 27). Taxonomic summaries and α and β diversity metrics, statistical analysis, and taxonomic classifications were computed using QIIME 2 software (28) and downstream analyses by R scripts were performed as previously reported (29). Linear discriminant analysis (LDA) effect size (LefSe) was used to compare fecal microbiome abundance profiles between treatment groups (30). The Huttenhower galaxy online platform was used to run LEfSe (http://huttenhower.sph.harvard.edu/galaxy/).

Following the primary microbiome analysis and noting that a subgroup of individuals had experienced significant flatulence (≥1 rating-point increase) during the PHF, we hypothesized that these individuals harbored organisms capable of fermenting PHF (fermenters) and, thus, may exhibit a microbiome profile different from the majority of the participants (nonfermenters). Subgroups were compared using LefSe analysis (30).

Statistical analysis

Sensory data for the any-age panels were collected using Compusense® Five software and older adults by paper ballots. Statistical analysis was carried out using Statistical Analysis Systems® 9.4 (SAS). A 2-factor ANOVA was performed to determine if there were differences in ratings for each attribute. Mean separation was completed using Duncan's multiple range test with an α level of 0.05.

The sample size of the trial was based on a power of 0.80 and a type I error rate of 0.05. Based on a study testing 10.5 g/d of an insoluble fiber (31), reporting a mean stool frequency per day for placebo of ∼1.00 and for treatment of 1.35 (range: 1.15–1.51) with SDs of 0.32 and 0.63, respectively, and a correlation of 0.35, a paired sample size of 26 for a crossover trial was needed to show a significant effect. Estimating a drop-out rate of 25%, a sample of 36 was targeted.

Daily symptoms, GSRS syndromes, and stool frequency were analyzed as intent-to-treat. Unless noted otherwise, data are presented as means ± SEMs. Significance was set at an α level of 0.05. Linear mixed models were used to test differences between treatment groups for the daily questionnaire symptoms, GSRS syndromes, SNAQ scores, and dietary intake. Data were square root transformed, where appropriate, with baseline as a covariate. When the *F*-value was significant, a multiple-means comparison was performed using Tukey–Kramer at a *P* value of 0.05. SNAQ was compared as the difference between means and categorically (risk: ≤14 points vs. >14 points). A 2-tailed Fisher's exact test was used to test the effectiveness of blinding. Paired *t* test was used to determine if fiber intake was different between the subgroups at baseline. For the microbiome data, the Kruskal-Wallis test was used to compare α diversities. QIIME software suite was used to calculate the metrics corresponding to diversity and taxonomic classification (28).

Results

Product development and sensory evaluation

Sensory panels of older adults (aged ≥60 y) evaluated the PHF and control cinnamon mixed berry ($n = 76$) and oatmeal raisin chocolate chip ($n = 74$) cookies as did 2 panels of all ages ($n = 120$/panel) in comparison to commercial cookie varieties. Results of the sensory evaluations are shown in **Supplemental Table 1**. Overall liking was rated highest

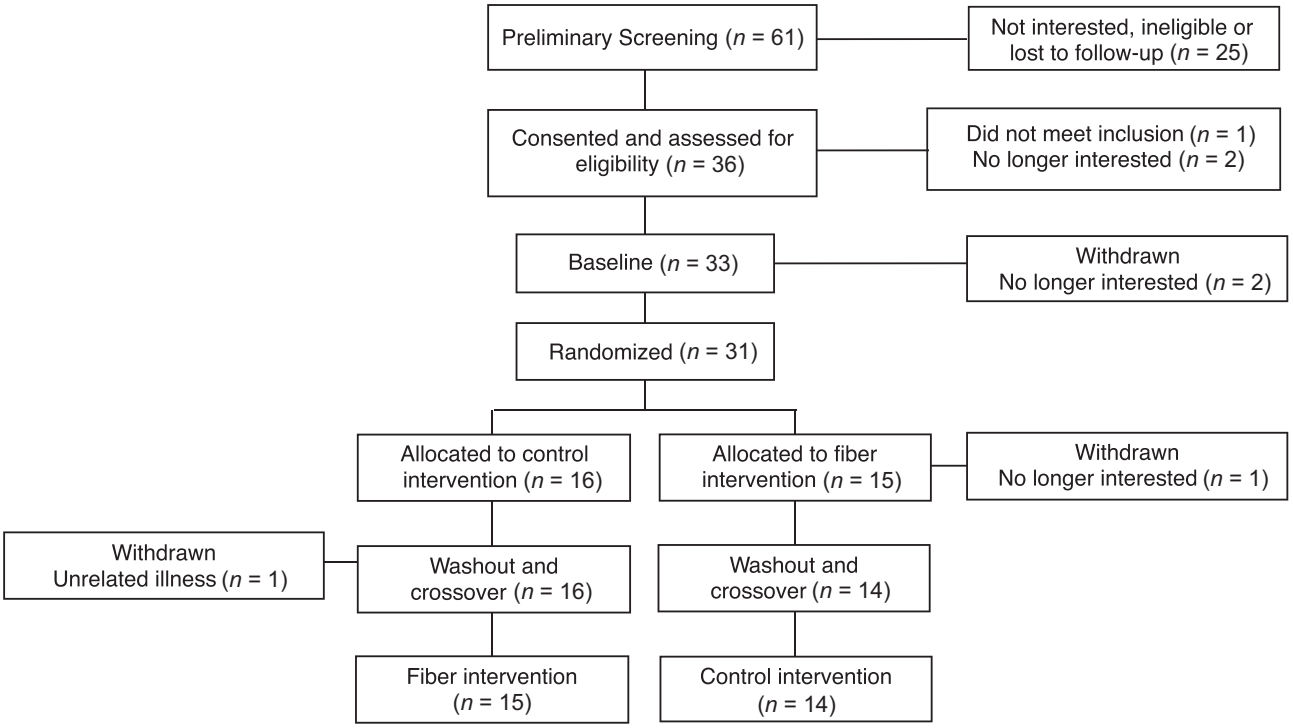

FIGURE 2 Participant recruitment, randomization, and study flow diagram.

for the FiberOne® sugar cookie and the control cinnamon mixed berry cookie. Belvita® and the cinnamon mixed berry with PHF were rated somewhat lower but similar. Overall liking for the FiberOne® oatmeal raisin cookie and the control oatmeal raisin chocolate chip cookie was not different. Belvita® and the oatmeal raisin chocolate chip cookie with PHF were rated as similar. The nutrient compositions of the PHF and control cookies evaluated for acceptability and subsequently used in the intervention trial are shown in **Supplemental Table 2**.

Wellness outcomes.

The intervention study flow diagram is presented as **Figure 2**. Participant demographics and characteristics are shown in **Table 1**. Of the 36 individuals who consented, 3 withdrew prior to baseline, 2 withdrew prior to randomization, 1 withdrew during the first intervention period (fiber), and 1 withdrew during the first washout. Participants were unable to ascertain whether they were consuming the PHF or control snacks (intervention 1, $P = 0.6$; intervention 2, $P = 0.3$), confirming the effectiveness of blinding. Participants reported normal stool frequency (1.63 ± 0.05 stools/d) at baseline, with no significant changes with interventions (**Table 2**). BSFS reporting showed 12.0% slow transit, 76.6% normal transit, and 11.4% fast transit at baseline, with no differences between periods (Table 2). No significant differences were reported for the GSRS (Table 2). For daily symptoms (Table 2), abdominal noises and bloating were higher for the PHF intervention compared with the control, and flatulence was higher for PHF compared with baseline, whereas there were no differences for daily abdominal cramping, constipation, diarrhea, or appetite. Mean SNAQ score was higher during PHF for the fiber period (15.8 ± 0.4) compared with the control (15.3 ± 0.4); however, when assessed as risk categories (at risk vs. no risk) the apparent difference was not significant (PHF: $n = 7$ at risk; control: $n = 10$ at risk). At baseline ($n = 29$), total energy intake was 1780 ± 179 kcal/d and did not differ between intervention periods. Similarly, background fiber (18.4 ± 2.0 g/d), protein (71.6 ± 7.6 g/d), carbohydrate (204.7 ± 21.6 g/d), and fat (72.7 ± 8.6 g/d) intakes reported at baseline did not change with the interventions. At baseline, the mean fiber intake of the subgroup of fermenters (18.6 ± 4.5 g/d) was not different from that of the nonfermenters (18.2 ± 2.5 g/d). With the inclusion of study snacks, there was a significant increase in total fiber intake during the PHF period ($P < 0.0001$). Intake of dairy foods, some of which may have contained live cultures, did not significantly differ between baseline (1.6 ± 0.3 servings/d) and interventions (PHF: 1.4 ± 0.1 servings/d; control: 1.4 ± 0.3 servings/d). Body weight did not change between periods or over the duration of the study period (data not shown).

Microbiota composition.

The relative abundances of bacteria observed for all participants during the baseline, washouts (pooled), control, and PHF are shown in **Figure 3** and as a Krona figure in **Supplemental Figure 1**. No differences were seen between periods with LefSe analyses. There was no change in bacterial α diversity with PHF (**Supplemental Figure 2**). For the subgroup analysis carried out to compare the microbiome of those participants demonstrating an increase in flatulence severity as an indicator of PHF fermentation, baseline differences between fermenters ($n = 7$) and nonfermenters ($n = 22$) are shown in **Figure 4**. Taxa such as *Methanobrevibacter*, *Coprococcus*, and Peptosteptococcaceae were higher in the fermenters compared with nonfermenters.

TABLE 1 Participant demographics and characteristics

	Participants ($n = 31$)	Fermenters[1] ($n = 7$)	Nonfermenters[2] ($n = 22$)
Gender (M/F), *n*	14/17	2/5	11/11
Age, mean ± SD (range), y	69.7 ± 6.5 (60–86)	68.6 ± 6.0 (61–80)	70.3 ± 6.9 (60–86)
Race, *n* (%)			
African American	4 (12.9)	2 (28.6)	2 (9.1)
White	27 (87.1)	5 (71.4)	20 (90.9)
Ethnicity, *n* (%)			
Hispanic	2 (6.4)	0	2 (9.1)
Non-Hispanic	27 (87.1)	6 (85.7)	19 (86.4)
Not reported	2 (6.4)	1 (14.3)	1 (4.5)
BMI (in kg/m^2), *n* (%)			
Normal (18.5–24.9)	8 (25.8)	0	8 (36.3)
Overweight (25–29.9)	9 (29.0)	3 (42.9)	6 (27.3)
Obese (>30)	14 (45.2)	4 (57.1)	8 (36.4)
Reported compliance[3]			
Pea hull fiber snacks	98%		
Control	96%		

[1]Participants demonstrating an increase of ≥1 point (scale 0 to 6) in flatulence severity reported in the daily questionnaire during the PHF intervention.
[2]Participants demonstrating a <1 point increase in flatulence severity during the PHF intervention.
[3]Daily questionnaire reporting.

Nonfermenters were enriched in Proteobacteria. **Figure 5** shows a significantly higher abundance of Clostridiales during the control compared with the PHF intervention in fermenters. No significant changes in microbiome profile were detected in the nonfermenter subgroup with the provision of PHF compared with control ($n = 22$). A comparison of the relative bacterial proportions for all samples from all periods for fermenters versus nonfermenters is shown in **Figure 6**; using a stringent (LDA = 3) cutoff, differences were demonstrated in numerous taxa. A comparison of baseline with washout demonstrated only an enrichment of *Lactobacillus* during baseline (**Supplemental Figure 3**).

Discussion

PHF-fortified snacks evaluated in this clinical trial were assessed as being acceptable by sensory panelists, including older adults. The study snacks provided ~200 kcal/d, but the intake of the snacks did not

TABLE 2 Daily and biweekly reported wellness outcomes[1]

	Baseline	Fiber	Control	*P*
Stool frequency				
Stools/d	1.63 ± 0.05	1.85 ± 0.05	1.76 ± 0.05	NS
BSFS, %				
Slow transit	12.0	9.2	11.3	NS
Normal transit	76.6	77.0	78.4	NS
Fast transit	11.4	13.8	10.3	NS
Daily symptom scores				
Appetite	4.07 ± 0.07	3.80 ± 0.07	3.94 ± 0.07	NS
Abdominal cramping	0.16 ± 0.03	0.23 ± 0.03	0.10 ± 0.02	NS
Abdominal noises	0.27 ± 0.03^{a,b}	0.37 ± 0.04^{a}	0.18 ± 0.02^{b}	<0.01
Bloating	0.26 ± 0.03^{a,b}	0.34 ± 0.04^{a}	0.18 ± 0.03^{b}	<0.05
Flatulence	0.74 ± 0.04^{b}	1.05 ± 0.05^{a}	0.83 ± 0.05^{a,b}	<0.05
Constipation	0.12 ± 0.02	0.16 ± 0.02	0.20 ± 0.04	NS
Diarrhea	0.17 ± 0.03	0.16 ± 0.02	0.12 ± 0.03	NS
GSRS syndrome scores				
Abdominal pain	1.21 ± 0.07	1.36 ± 0.11	1.24 ± 0.12	NS
Reflux	1.09 ± 0.05	1.07 ± 0.04	1.09 ± 0.04	NS
Indigestion	1.41 ± 0.08	1.59 ± 0.13	1.40 ± 0.09	NS
Constipation	1.24 ± 0.10	1.13 ± 0.04	1.29 ± 0.10	NS
Diarrhea	1.25 ± 0.07	1.24 ± 0.06	1.43 ± 0.15	NS

[1]Values are means ± SEs unless otherwise indicated. Stool form were categorized into slow transit (types 1 and 2), normal transit (types 3 to 5), and fast transit (types 6 and 7). Means with different superscript letters differ significantly according to Tukey-Kramer ($P < 0.05$). BSFS, Bristol Stool Form Scale; GSRS, Gastrointestinal Symptom Response Scale.

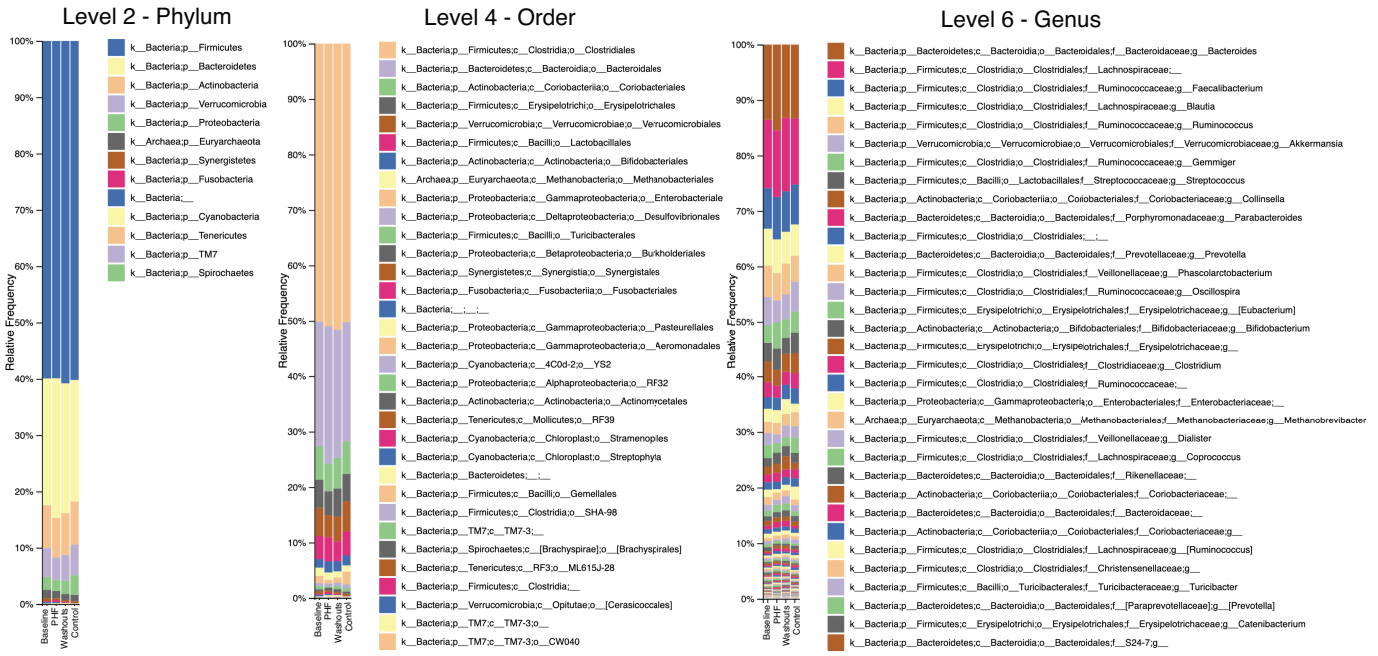

FIGURE 3 Relative abundance of bacteria by phyla, order, and genus levels observed during baseline, PHF, washouts (pooled), and control periods. PHF, pea hull fiber.

result in differences in energy intake during the intervention periods, suggesting participants substituted instead of adding study foods to their usual diet. Appetite as assessed by SNAQ category (23) did not change during the PHF intervention, suggesting no increased risk of unintended future weight loss in this older cohort; and the daily reporting of appetite was similarly unaffected. In most previous studies, consuming PHF-fortified foods did not affect appetite, food intake, or body weight (18, 32–34). In contrast, a study in overweight/obese adults

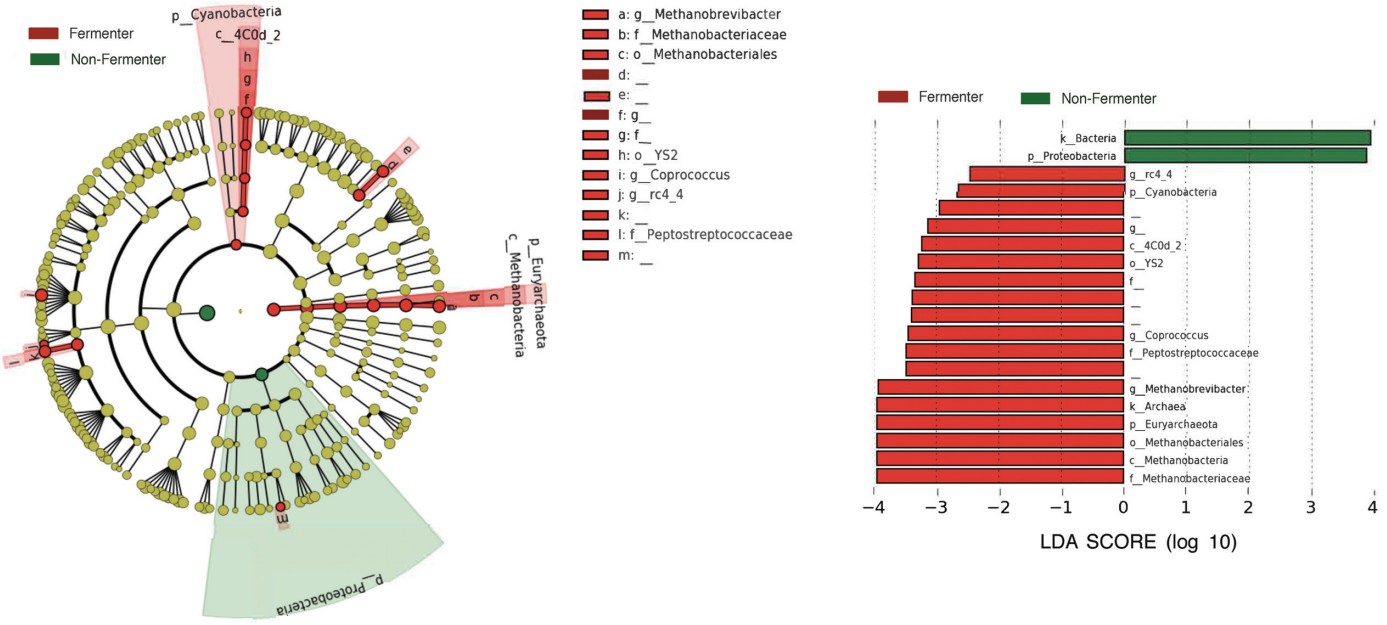

FIGURE 4 LDA effect size (LefSe) comparing the relative bacterial proportions. Fermenters (participants responding to PHF intake with an increase in flatulence severity) ($n = 7$) versus nonfermenters at baseline ($n = 22$). c, class; f, family; g, genus; k, kingdom; LDA, linear discriminant analysis; o, order; p, phylum; PHF, pea hull fiber.

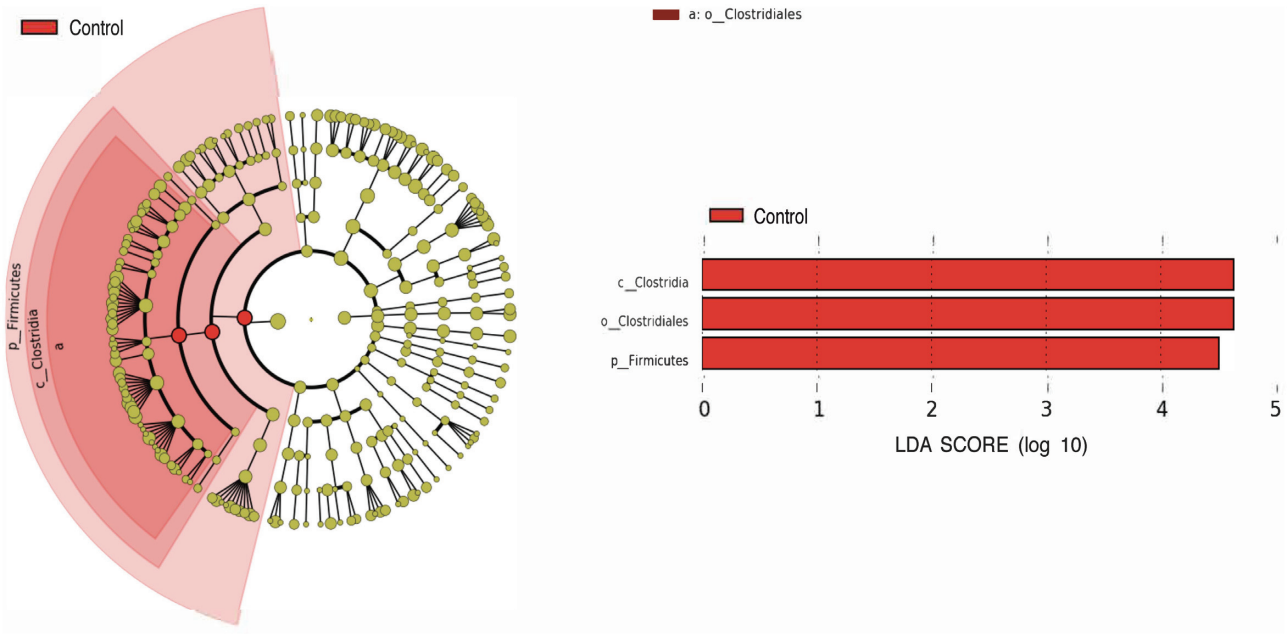

FIGURE 5 LDA effect size (LefSe) comparing the relative bacterial proportions in the subgroup of fermenters (participants responding to PHF intake with an increase in flatulence severity) ($n = 7$) during PHF versus control periods. c, class; LDA, linear discriminant analysis; o, order; p, phylum; PHF, pea hull fiber.

(aged 44 ± 15 y) showed weight loss with the consumption of 15 g/d of PHF in the form of wafers over 12 wk and decreased food intake in a single-meal study (35). However, appetite and sensory acceptance were not reported and, thus, it is possible that the wafers were somewhat unappetizing and, as such, food intake may have been negatively impacted independent of any metabolic effect resulting from any PHF fermentation. It may also be possible that age confounds appetite response to foods with added fiber. As SNAQ is validated to predict the future risk of weight loss (23) and poor outcomes in older adults (36), this tool may be useful to assess the appropriateness of added fibers for supplementation of foods intended for older adults. PHF did not suppress appetite; thus, it may be an appropriate fiber for fortification of foods for older adults at risk of unintended weight loss.

Reported GSRS syndrome scores fell below clinical significance (mild discomfort) for participants overall, suggesting they were healthy with respect to gastrointestinal function. Stool form was used as a proxy for transit time, and participants reported primarily normal transit stools at baseline, which remained unchanged with PHF. Participants reported the proportion of slow transit stools at 11%, somewhat higher than a representative sample of the US population considered to have normal bowel habits at 6% slow transit (37), but lower than we have previously reported in younger women (38). The PHF used in this study was finely processed (200 mesh) and, thus, its high surface area compared with intact hulls may have facilitated fermentation by the colonic bacteria, thus lessening its laxative effects, a mechanism that has been previously suggested (39). It has been reported that coarse wheat bran decreased transit time, whereas the same dose of finely ground wheat bran did not (40). Similarly, finely ground oat hull fiber did not impact transit time in young male subjects with relatively fast transit time (41). Although stool form is strongly associated with microbiota profile (19), the changes in the microbiota observed in some of the participants in the present study are likely due to the fermentation of PHF rather than an effect of altered transit time.

Although the current US FDA considers increased stool frequency as a "beneficial physiological effect on human health" for isolated or synthetic nondigestible carbohydrates (functional fibers) (42), in healthy individuals with normal stool frequency, fibers, fermentable fibers in particular, may not alter laxation (39). Although in the present trial an insoluble dietary fiber versus functional fiber was examined, stool frequency did not change. This finding may not be unexpected given that the participants exhibited normal stool frequency (37) at baseline and displayed no symptoms of constipation. In addition, in nonconstipated individuals, stool frequency is not associated with transit time (43). The results of this study add to the significant literature that refutes stool frequency as an appropriate outcome for the evaluation of fiber supplementation in individuals with normal bowel habits. As increases in stool frequency are more often seen as a response to fiber in those with infrequency (e.g., <3 stools/wk) (44) and functional constipation (45), it is possible that if PHF was tested in community-dwelling older adults with low baseline stool frequency and symptoms of constipation, an increase may have been seen, as has been demonstrated in older adults residing in long-term care (18). Similarly, in a small study in older adults with chronic kidney disease, stool frequency increased with an intake of 10 g/d of PHF (46). These conflicting findings may, in part, be due to the length of the intervention. The studies that have shown an increase in frequency with PHF tested it with intervention periods of 4 wk versus 2 wk in the present trial. Further, differences in resident microbiota, extent of fermentation, and transit time may have affected

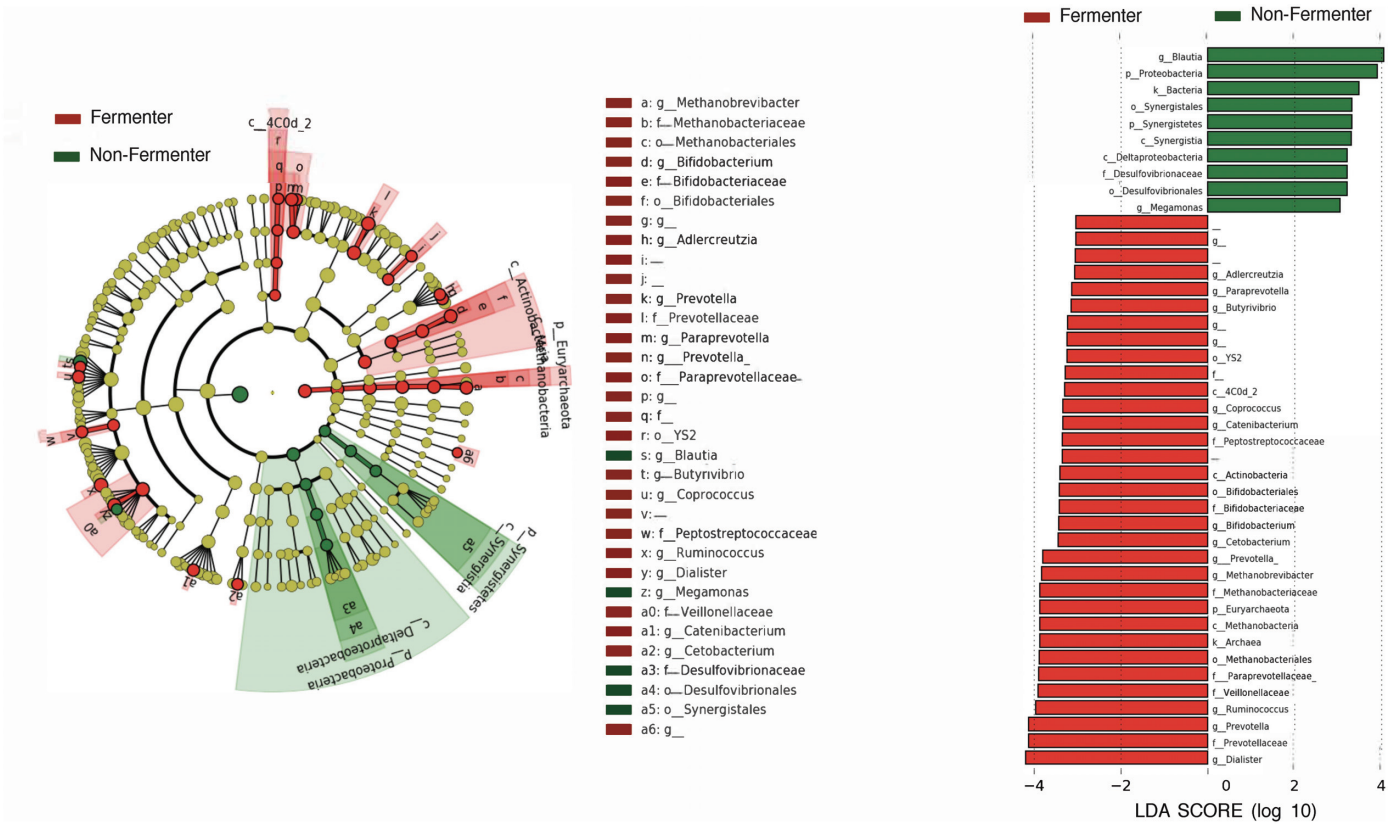

FIGURE 6 LDA effect size (LefSe) comparing the relative bacterial proportions for all samples from all periods in the subgroup of fermenters ($n = 7$) (participants responding to PHF with an increase in flatulence severity) versus nonfermenters ($n = 22$). c, class; f, family; g, genus; k, kingdom; LDA, linear discriminant analysis; o, order; p, phylum; PHF, pea hull fiber.

the outcomes. Beyond stool frequency, quantitative measurements such as stool bulk and examination of in vivo fermentability require exploration.

The participants' baseline microbiota showed significant intraindividual variation, which was not surprising given the diversity that has been shown in the human microbiome (47) and its response to differing dietary patterns (9). The results of this study did not provide evidence for PHF as a means to increase microbial diversity or any modulation of microbiota in most participants. It has been suggested that a higher-fiber diet may not have remarkable effects on the microbiome profile in the short term (48) unless an extreme change is made in dietary intake, such as the elimination of fiber and other carbohydrates from the diet (49). A longer-term exposure to the fiber source may be necessary to significantly impact microbiota. However, in the present study, a subgroup analysis revealed significant differences in the microbiota of those individuals exhibiting increased flatulence (fermenters), suggesting fermentation of PHF by gas-generating microorganisms. *Methanobrevibacter*, *Coprococcus*, and Peptostreptococcaceae were higher in fermenters. A higher abundance of methanogens, primarily *Methanobrevibacter*, may be unfavorable as methane production has been associated with constipation (50); however, it has been suggested that certain methanogens, at least theoretically, may suppress trimethylamine production and, thus, that of trimethylamine-*N*-oxide, which is associated with cardiovascular disease risk (51). Legume intake has been shown to depress proteolytic bacteria (52). Consuming snacks containing PHF resulted in decreased abundance of Clostridiales compared with controls in the fermenters subgroup, specifically a suppression of *Clostridia*, which are implicated in protein fermentation (53). Recently, a suppression of unclassified Clostridiales after feeding vegetables high in fructans (artichokes and leeks) was reported (54). In contrast, feeding 15 g/d of PHF over a 12-wk period showed no treatment effect on the limited number of taxa evaluated (33). In a secondary analysis, *Lachnospira* increased with PHF (55). However, as subjects also lost weight in the PHF intervention arm, it is possible that the change in *Lachnospira* was due to weight loss instead of the PHF specifically, as a similar enrichment has been seen in bariatric surgery (56) and nonalcoholic fatty liver disease patients (57) following weight loss. Increased *Bifidobacterium* and *Lactobacillus* have been commonly reported in fiber studies, but most often in response to oligosaccharide (e.g., galacto-oligosaccharide, fructo-oligosaccharide) supplementation (8) versus a complex fiber source with cellulose as the major indigestible polysaccharide constituent, such as PHF (58). We saw no changes in these genera. Diets higher in fiber, including legumes, support diversity of gut microbiota and these diets are associated with enhanced Bacteroidetes, specifically the genera *Prevotella* (59, 60). In addition, consumption of a Mediterranean diet has been associated with increased *Prevotella* (61). In the present study, no enhancement of *Prevotella* was seen with the PHF, although the subgroup of fermenters exhibited higher relative abundance of *Prevotella*, which may reflect

habitual higher fiber intake and possibly legume intake. However, the baseline fiber intakes of the fermenters did not differ from those of nonfermenters and long-term intake was not examined.

This study had limitations. As other research has shown, it is difficult to demonstrate change and conclude improvement in gastrointestinal function in individuals with normal bowel habits (62). The length of the intervention period may have been a limitation. Administration of fiber over many weeks or months, although not necessarily expected to impact stool frequency or form, may modulate microbiota. However, the comparisons of microbiome profiles seem to confirm the adequacy of the 2-wk washouts as they differed from baseline only by *Lactobacillus* abundance.

Current fiber intake in the United States falls below recommendations (63), a concern given that low fiber intakes are strongly associated with increased chronic disease risk (64, 65). Foods with added fiber may improve intakes (1); however, given the high burden of gastrointestinal symptoms in the United States (66), added fibers that do not substantially contribute to gastrointestinal discomfort are needed. The findings of the present study using snacks fortified with PHF, a primarily insoluble dietary fiber, showed that only a minority of individuals experienced an increase in daily symptoms of flatulence, bloating, and abdominal noise, suggesting differential fermentation or perhaps differing sensitivity to gas production. Thus, in older adults with normal bowel habits, PHF at 10 g/d is well tolerated. Future trials should test PHF in individuals with low stool frequency and slow transit stool form. Snacks fortified with PHF did not suppress appetite; thus, PHF may be an appropriate fiber source for older adults at risk of unintended weight loss and subsequent malnutrition.

It was suggested recently that controlled-feeding trials are needed to determine the specific effects of fiber sources on microbiome composition (9). We have shown that the baseline microbiome profile, particularly the predominance of methanogens, may be predictive of gastrointestinal symptom response to, and potentially health benefits of, PHF. It is not known if microbiome profile also may be predictive of response to whole pulses and other high-fiber ingredients. As has been suggested, individuals may exhibit individualized bacterial population responses to foods (9), and fiber specifically, and our results support this premise. The results of the present study confirm that baseline microbiome profile confounds symptom outcomes and, thus, also may influence health outcomes related to a fiber source. Further research is needed to explore the potential health effects of *Clostridia* suppression. As the impact of fiber on health is thought to be largely related to its modulation of microbiota and its metabolism (67), and possibly transit time (19), the effects of added fibers on these outcomes in community-dwelling older adults presenting with constipation and dysbiosis, specifically low baseline diversity, require investigation.

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