Effects of tempe smoothie on fecal scfas in perimenopausal women: a quasi-experimental pilot study

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Abstract. Tempe is a fermented soybean product rich in dietary fiber and bioactive compounds that may influence gut microbial fermentation and short-chain fatty acid (SCFA) production. This study aimed to evaluate the effects of tempe smoothie consumption on fecal SCFA concentrations in perimenopausal women. A quasi-experimental one-group pre–post intervention study was conducted in East Jakarta, Indonesia. Sixteen participants were involved, and eight with complete fecal samples were included in the final analysis. Participants consumed tempe smoothie (600 mL/day) five days per week for six weeks. Fecal SCFAs (acetate, propionate, butyrate, and valerate) were analyzed using gas chromatography–flame ionization detection (GC-FID). Paired tests were used to compare pre- and post-intervention values. Acetate proportion, absolute butyrate concentration, and total SCFAs increased after the intervention, although these changes were not statistically significant ($p>0.05$). In contrast, valerate proportion decreased significantly ($p=0.029$). In this pilot, uncontrolled study, tempe smoothie consumption was associated with directionally favorable changes in fecal SCFA profiles, suggesting a potential shift toward more beneficial microbial fermentation. These findings are exploratory and require confirmation in larger randomized controlled trials.

1. Introduction

The menopausal transition involves endocrine and metabolic changes that increase the risk of cardiometabolic disorders, inflammation, and gut dysbiosis. Declining estrogen levels during perimenopause are linked to alterations in immune function, metabolic regulation, and gut microbiota, highlighting the gut estrogen axis as a bidirectional interaction between host hormones and microbial activity [1, 2].

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Gut microbial metabolites, particularly short-chain fatty acids (SCFAs) acetate, propionate, and butyrate, play key roles in intestinal integrity, immune regulation, and metabolic processes. These metabolites are produced through fermentation of dietary fibers, suggesting that diet can influence gut microbial function and host physiology [3, 4].
Fermented foods such as tempe offer a promising approach, as they provide fermentable substrates and bioactive compounds, including isoflavones. These compounds may enhance SCFA production and interact with estrogen metabolism via microbial conversion to bioactive metabolites such as equol [5–10].

However, evidence from human intervention studies remains limited, particularly regarding tempe-based beverages in perimenopausal women. Therefore, this study evaluated the effects of tempe smoothie consumption on fecal SCFA profiles (acetate, propionate, butyrate, and valerate) as markers of gut microbial fermentation.

2. Materials and methods

2.1 Study design, setting, and participants

This study employed a quasi-experimental one-group pre–post intervention design. It was conducted in Bambu Apus, Cipayung District, East Jakarta, Indonesia. Participants were recruited through community health centers and local announcements. Sixteen perimenopausal women were initially enrolled based on predefined eligibility criteria.

Inclusion criteria were women aged 45–55 years in the perimenopausal stage who were willing to participate and able to comply with the study procedures. Exclusion criteria included major chronic diseases, gastrointestinal disorders that affect absorption, and recent use of hormone therapy, antibiotics, or probiotic/prebiotic supplements.

All participants provided written informed consent. The study protocol was approved by the Health Research Ethics Committee of Universitas Respati Indonesia (No. 579/SK.KEPK/UNR/VIII/2024) and the study was conducted in accordance with the Declaration of Helsinki.

During the study, attrition occurred. Of the 16 enrolled participants, 8 were excluded from the final SCFA analysis due to incomplete fecal sample collection (n=3), non-adherence (n = 2), and withdrawal or loss to follow-up (n=3). Consequently, complete fecal SCFA data were available for 8 participants and included in the final analysis.

2.2 Tempe smoothie preparation and intervention

Tempe used in this study was obtained from a certified local producer in Jakarta operating under Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Point (HACCP) standards, using *Rhizopus oligosporus* starter culture.

To ensure consistency, tempe was prepared in weekly batches, stored at 4°C, and used within 48 hours. Each serving was prepared using a standardized protocol: 100 g of fresh tempe was diced and blanched in boiling water for 5 minutes to reduce microbial load and improve sensory acceptability. The blanched tempe was then blended with 600 mL of distilled water using a high-speed blender to produce a homogeneous smoothie. No additional ingredients were added.

Isoflavone content (genistein and daidzein) was quantified using high-performance liquid chromatography (HPLC) following established analytical methods [8], with each 600 mL serving providing approximately 37 mg total isoflavones. Batch-to-batch variability was controlled by analyzing one sample per batch, with acceptable variation <10%.

The study included a one-week run-in period followed by a six-week intervention phase. Participants consumed 600 mL of tempe smoothie once daily, five days per week. Compliance was monitored through daily consumption logs, weekly follow-up visits, and return of empty containers. Adherence was calculated as the percentage of consumed doses relative to the prescribed intake.

2.3 Data collection and laboratory analysis

Baseline and post-intervention measurements focused on fecal SCFA concentrations as primary biochemical markers. Participants were instructed to maintain their habitual diet and avoid fermented foods, probiotics, and antibiotics during the study period.

Fecal samples were self-collected using sterile biohazard containers and transported to the laboratory within 4 hours under cooled conditions (4°C). Samples were homogenized using anaerobic diluent to ensure uniformity.

SCFA concentrations (acetate, propionate, butyrate, and valerate) were measured using gas chromatography with flame ionization detection (GC-FID) based on established protocols [3, 4], with minor modifications. All samples were analyzed in blinded duplicate. Calibration curves were prepared using certified SCFA standards (linear range 0.1–20 mmol/L; $R^2>0.99$), and quality control included reference standards and internal controls in each analytical run.

2.4 Statistical analysis

Normality was assessed using the Shapiro–Wilk test. Normally distributed data were analyzed using paired t-tests, while non-normally distributed data were analyzed using the Wilcoxon signed-rank test. Results are presented as mean±standard deviation (SD). Statistical significance was defined as $p<0.05$.

3. Results and discussion

3.1 Results

A total of sixteen participants were enrolled in the study; however, complete fecal SCFA data were available for eight participants and were included in the final analysis. This reduction from the initial sample may affect statistical power and should be considered when interpreting the findings.

At baseline, participants had a mean age of 54±3.2 years and a mean body mass index (BMI) of 26.4±4.7 kg/m², indicating that most participants were in the overweight category. These characteristics provide important context for interpreting metabolic and microbial outcomes in this population.

Overall changes in fecal SCFA profiles before and after the intervention are presented in Table 1. In brief, there was a non-significant increase in acetate proportion, absolute butyrate concentration, and total SCFA levels following tempe smoothie consumption ($p>0.05$). In contrast, the proportion of valerate decreased significantly after the intervention ($p=0.029$).

Table 1. Changes in Fecal Short-Chain Fatty Acid (SCFA) profiles from baseline to post-intervention (n=8).

SCFA Component	Baseline (Mean±SD; min–max)	Post-Intervention (Mean±SD; min–max)	Mean Difference (Post–Baseline)	Test Statistic (t)	p-value
Acetate (%)	51.50±4.57 (45–59)	55.75±5.01 (48–61)	+4.25	-1.73	0.127
Propionate (%)	23.63±6.91 (13–32)	22.88±7.68 (17–40)	-0.75	0.27	0.795
Butyrate (%)	17.50±4.75 (8–22)	16.75±4.33 (9–22)	-0.75	0.32	0.762
Valerate (%)	3.38±0.91 (1.6–4.6)	1.96±0.84 (0.4–3.0)	-1.42	2.74	0.029*
Butyrate (mg/mL)	3.65±1.50 (1.3–5.7)	3.88±1.59 (2.3–6.4)	+0.23	-0.23	0.828
Total SCFA (mg/mL)	16.63±5.63 (9–25)	18.13±5.38 (11–27)	+1.50	-0.45	0.663

Notes: Values are presented as mean±SD with minimum–maximum range in parentheses. Mean differences are calculated as post-intervention minus baseline. Statistical significance was set at $p < 0.05$. *$p < 0.05$.

3.2 Discussion

This study suggests that tempe smoothie consumption may modestly influence fecal SCFA profiles in perimenopausal women. Baseline characteristics particularly older age and overweight BMI are relevant, as both factors are known to influence gut microbiota composition and SCFA production, potentially contributing to the variability and modest effect sizes observed.

Although most changes were not statistically significant, the observed increases in acetate, butyrate, and total SCFAs may indicate a shift toward enhanced saccharolytic fermentation. This is biologically plausible, as dietary fibers and oligosaccharides in fermented soy products serve as substrates for microbial fermentation, leading to SCFA production [3,4]. Acetate and butyrate play key roles in energy metabolism, intestinal barrier function, and immune regulation [3], suggesting that even modest increases may reflect functionally relevant changes.

The significant reduction in valerate may indicate decreased proteolytic fermentation, which is typically associated with less favorable metabolic by-products [4]. This pattern may reflect a shift toward a more favorable microbial metabolic profile; however, causal interpretation is limited by the study design.

Serum folate was initially included as a biochemical marker due to its role in one-carbon metabolism and potential interaction with gut microbiota. However, no meaningful changes were observed following the intervention (data not shown), suggesting that the intervention duration or dose may have been insufficient to influence systemic folate status.

These findings may be partly explained by the composition of tempe, which provides fermentable substrates and bioactive compounds, including isoflavones [5–7]. Fermentation enhances nutrient bioavailability and may support microbial metabolism in the colon [5]. Although isoflavones can be metabolized into more bioactive compounds such as equol [10],

this study did not directly assess these pathways; therefore, mechanistic interpretations remain speculative.

Several limitations should be acknowledged. The small sample size (n=8) with complete data) reduces statistical power and may introduce selection bias due to participant attrition. Additionally, the absence of a control group limits causal inference. Accordingly, these findings should be interpreted as exploratory rather than confirmatory.

4. Conclusion

In this pilot, uncontrolled study, tempe smoothie consumption was associated with directionally favorable changes in fecal SCFA profiles, including increases in acetate, butyrate, and total SCFAs, along with a significant reduction in valerate. These findings are consistent with a possible shift toward more beneficial carbohydrate-driven microbial fermentation.

Given the small sample size and lack of a control group, these results should be interpreted as exploratory and hypothesis-generating rather than confirmatory. The observed patterns are biologically plausible and may reflect a modulatory effect of tempe-derived fermentable substrates on gut microbial metabolism.

From a practical perspective, these findings suggest that incorporating fermented soy-based foods such as tempe into the diet may represent a feasible nutritional approach to support gut microbial activity in perimenopausal women, although evidence remains preliminary.

Future studies using randomized controlled designs, larger sample sizes, and integrated microbiome and metabolic analyses are needed to confirm these findings and clarify their implications for gut health and metabolic regulation in perimenopausal women.

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