

An Investigation into the Effectiveness of Kefir in Managing Stress and Anxiety in College Students. A Randomised Controlled Trial with Assessment of Nutritional Status.

Colm Nolan, Lorna Doyle and Bruce Wardrop, South East Technological University

Abstract

With anxiety and stress becoming increasingly prevalent, the need for practical interventions has grown. Nutritional psychiatry has explored probiotics, omega-3, vitamin D and fibre in managing mental health via the gut-brain axis. This paper aimed to determine the effectiveness of kefir in managing anxiety, stress and gastrointestinal (GI) health. It also examined the relationship between omega-3, vitamin D and fibre intake, and these outcomes, as well as the influence of GI health on anxiety and stress. Sixteen participants were assigned to an intervention (kefir) or control (rice milk) group and consumed 250 ml of their allocated drink for 14 consecutive days. Anxiety, stress, GI health and dietary intake were measured pre- and post-intervention. Findings showed that anxiety significantly reduced in both the kefir (Mean difference = 2.5, $p = 0.033$) and control groups (mean difference = 4.5, $p = 0.005$), with no significant difference between groups ($p = 0.197$). There were no significant changes in stress or GI health. Baseline GI health was not significantly correlated with anxiety or stress. Nutrient intake had no significant associations with anxiety, stress or GI health. Therefore, kefir did not reduce anxiety or stress compared with the control condition. Future research should include longer trials, larger sample sizes and biomarker use.

Keywords: Effect of kefir on anxiety, Kefir and perceived stress, Probiotics and Anxiety in College students, Impact of gut health on anxiety, Gut-brain axis, Mental health, Nutritional psychiatry in healthy adults

Introduction

Stress and anxiety disorders have become such a prevalent part of our existence (Goodwin *et al.*, 2020: 443), and the need for accessible interventions has never been greater. While medicinal approaches have remained a source of support for managing such disorders, a strategy in the form of nutritional psychiatry has emerged (Norwitz and Naitoo, 2021). With that, an emphasis has been placed on the **gut-brain axis**.

The gut-brain axis has been linked to the regulation of anxiety and stress (Lee and Kim, 2021: 3; Morys *et al.*, 2024). Anxiety is a multidimensional and often misunderstood psychological condition. Conceptually, anxiety can arise from the belief that one is under threat or danger (Gouveia, 2022: S671), becoming a disorder if these beliefs persist for more than 6 months (Stein and Sareen, 2015: 2059). Stress, on the other hand, is described as a state of worry or mental tension caused by a difficult situation, while chronic stress has been linked to the development of anxiety disorders (Khan and Khan, 2017). Anxiety and stress may also be influenced by alterations in gut microbial composition, which have been associated with changes in dopamine and serotonin signalling (Wilson *et al.*, 2024: 80–82). Similarly, inflammation (Spaeth *et al.*, 2018: 397) and lower **gut microbiome diversity** (Huang *et al.*, 2022: 398) have been associated with greater symptoms of stress and anxiety. This association underscores the importance of understanding how the gut **microbiota** develops and interacts with psychological functioning.

Gut microbiota diversity is initially shaped through variables in early life such as infection, antibiotic use, host genetics and mode of birth delivery (Cryan *et al.*, 2019: 1904). Thereafter, dietary patterns play an important role in gut microbiota diversity and gut-brain axis functionality (Roy, 2024). **Probiotic** intake represents one such dietary strategy for modulating the gut microbiota. Probiotics are defined as living microorganisms that provide a health benefit to the host when consumed in sufficient quantities (Hill *et al.*, 2014: 506). In a randomised controlled trial of healthy pregnant women, probiotic supplementation increased the functional (metabolic) diversity of the gut microbiome (Ma *et al.*, 2024). The gut microbiota is also involved in the production of neurotransmitters such as serotonin and dopamine, which are implicated in mood regulation (Al-Doghaither *et al.*, 2023). Additionally, a systematic review and

meta-analysis found that probiotic supplementation may reduce **cortisol**, a key stress hormone, including in healthy populations (Jain *et al.*, 2024).

Probiotics may therefore offer a promising, non-invasive avenue for supporting mental health within nutritional psychiatry. However, probiotics are distinct from fermented foods, which are foods produced through desired microbial growth and the enzymatic conversion of food components (Marco *et al.*, 2021: 196). Although many fermented foods contain live microorganisms, the presence of live cultures does not in itself indicate probiotic activity. Fermented foods only qualify as probiotics where there is evidence that the microorganisms they contain are delivered at an adequate level and confer a health benefit in humans (Hill *et al.*, 2014: 511).

Kefir is a dairy product derived from the fermentation of kefir grains in milk. Kefir contains a diverse symbiotic community of bacteria and yeasts that provides an optimal environment for the development of bacteria, which could elicit positive effects. These communities contain multiple strains of ***Saccharomyces***, ***Leuconostoc***, ***Bifidobacterium*** and ***Lactobacillus***, which may offer health benefits for the host (Kim *et al.*, 2019: 1784). However, when kefir itself was examined in a single-arm trial of women with polycystic ovary syndrome (PCOS), eight weeks of daily 250 ml consumption did not significantly alter **alpha diversity** or **beta diversity** of the gut microbiota. Nevertheless, participants reported significant improvements in the mental health domain of the SF-36 quality-of-life questionnaire (Çıtar Dazıroğlu *et al.*, 2024: 5638–39). Kefir has also enhanced positive affect in overweight adults relative to milk (Pražnikar *et al.*, 2020: 2966), although reductions in depressive symptoms did not differ from the control group in adults with non-alcoholic fatty liver disease (Mohsenpour *et al.*, 2023: 5). In healthy adults, kefir reduced gastrointestinal (GI) symptoms without significantly improving mental well-being (Bakırhan *et al.*, 2025: 5) and did not significantly alter anxiety, depression or stress markers (Cannavale *et al.*, 2023: 269). Consequently, its effects on anxiety and stress in healthy populations remain unclear.

Beyond probiotics and fermented foods, several individual nutrients, including omega-3 fatty acids (Zinkow *et al.*, 2025), vitamin D (Cheng *et al.*, 2020: 553; Singh *et al.*,

2020) and dietary fibre (La Torre *et al.*, 2021), have been independently linked to both the gut microbiota and mental health outcomes.

Omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have anti-inflammatory properties that may counteract the adverse effects of inflammatory stress exposures on the gut (Fu *et al.*, 2021: 4). These stressors may influence gut microbial composition and intestinal epithelial damage (Fu *et al.*, 2021: 4; Pai *et al.*, 2020: 258), and omega-3 intake has been linked to improved gut function and intestinal barrier integrity (Kawamura *et al.*, 2023: 198–200). Omega-3 intake may also play a role in managing psychological well-being (Kumarasivam *et al.*, 2022). It acts by lowering inflammatory markers, like **IL-1 β** , which are elevated in individuals with stress- and anxiety-related disorders; therefore, a reduction in these markers may result in improved stress and anxiety outcomes (Polokowski, 2019).

Vitamin D has multiple physiological roles, including anti-inflammatory effects within the gut and neuroregulatory functions relevant to mental health. Within the gut, vitamin D has been shown to alter microbiota composition, including the **Firmicutes-to-Bacteroidetes ratio**, with associated reductions in inflammatory markers (Garcia *et al.*, 2020; Singh *et al.*, 2020). In relation to mental health, a systematic review and meta-analysis found that vitamin D supplementation may reduce negative emotions (Cheng *et al.*, 2020: 553). Furthermore, in individuals with psychiatric conditions, vitamin D supplementation reduced C-reactive protein (CRP), an inflammatory marker associated with chronic stress and anxiety (Jamilian *et al.*, 2019: 8). Additionally, vitamin D plays a role in the regulation of serotonin (Alimohammadi-Kamalabadi *et al.*, 2024). Serotonin is known for its role in regulating mood and mental health disorders (Albert and Blier, 2023: E400).

Finally, dietary fibre is a major determinant of gut microbiota composition. A systematic review of intervention trials in healthy adults found that fibre altered gut microbiota composition, with effects dependent on the dose, type and structure of the fibre consumed (Vinelli *et al.*, 2022: 7–8). Similarly, a systematic review and meta-analysis of randomised controlled trials in healthy adults found that fibre interventions increased faecal *Bifidobacterium* and *Lactobacillus* abundance (So *et al.*, 2018: 969). Fibre fermentation also increases faecal short-chain fatty acid concentrations, which

reduce inflammation and support intestinal health (Vinelli *et al.*, 2022). These mechanisms may also support mental health by regulating gut hormones associated with psychological well-being (La Torre *et al.*, 2021). In an observational study, higher dietary fibre intake was associated with up to 33 per cent lower odds of anxiety and 29 per cent lower odds of psychological stress (Esmailzadeh *et al.*, 2021).

The evidence presented suggests that these nutrients enhance gut health and may subsequently improve anxiety and stress levels. These coinciding benefits reinforce the importance of including these nutrients as part of a diet for both psychological well-being and GI function.

The interplay that exists between GI health and mental health disorders represents an opportunity for the development of innovative approaches to managing related conditions. Although evidence highlights the potential for probiotics and certain nutrients that enhance the gut-brain axis to improve mental health outcomes, this opportunistic avenue is not exempt from significant research gaps.

Human trials examining kefir and mental health outcomes remain scant, with much of the existing evidence derived from animal models (Silva *et al.*, 2023: 412). Syntheses of research on gut alterations and anxiety have similarly drawn on animal-based trials (Simmons *et al.*, 2020: 431). Where human trials of kefir have been conducted, they have often involved clinical populations (Çıtar Dazıroğlu *et al.*, 2024; Mohsenpour *et al.*, 2023), whereas the present trial was conducted in healthy adults.

In addition, other research has determined the efficacy of probiotics in pregnant women, which is an inherently specific population (Ma *et al.*, 2024). Omega-3 has been shown to alter gut microbiota composition; however, the population consisted of individuals with ulcerative colitis (Prossomariti *et al.*, 2017: 7). Similarly, research examined vitamin D and its role in influencing the gut-brain axis; however, these studies were based on outcomes in participants with psychiatric conditions (Alimohammadi-Kamalabadi *et al.*, 2024; Jamilian *et al.*, 2019: 2). Although promising, these findings limit generalisability, proving difficult to translate to a population with non-psychiatric disorders and no GI issues.

A systematic review and meta-analysis of 34 controlled clinical trials found that probiotics produced small but significant improvements in symptoms of depression and anxiety (Liu *et al.*, 2019: 13). However, while an overall benefit of probiotics on mental health outcomes has been observed, findings for anxiety tend to be weaker than for depression (Asad *et al.*, 2025). Although these findings present a case for managing mental health through probiotic intake, the influence of probiotics in independently managing anxiety and stress, rather than as part of broader or combined mental health interventions, remains underexplored.

This paper aims to address these gaps by examining the independent effects of kefir on anxiety, stress and GI health in a healthy adult population. This research will also examine the impact of omega-3, vitamin D and fibre on the same variables. Finally, the role of gastrointestinal health in the development of anxiety and stress will be examined.

This research aligns with the broader field of nutritional psychiatry, which examines the biological mechanisms linking diet and mental health, including the gut-brain axis and the role of dietary interventions in both prevention and treatment (Marx *et al.*, 2026: 2). By examining kefir and specific nutrients in a healthy population, this study explores their potential as preventative, diet-based strategies for managing anxiety and stress. The insights gathered may inform future research aimed at developing accessible dietary-based interventions.

Methodology

Design

A randomised controlled trial was implemented to determine the effectiveness of kefir in managing stress and anxiety. An additional focus was placed on the assessment of nutritional status (omega-3, vitamin D and fibre) and GI symptoms.

The intervention was carried out over 14 days. The participants, at a 1:1 ratio, were randomly assigned to either the intervention group or the control group. To ensure random distribution, participants were assigned using computer software (gigacculator.com). The respective groups were instructed to consume 250 ml (Çıtar

Dazıroğlu *et al.*, 2024: 5633–43) of Biotiful kefir yoghurt drink or 250 ml of Joya rice milk for 14 consecutive days. Biotiful kefir is a fermented dairy drink made from pasteurised cow's milk and live kefir cultures, including *Bifidobacterium*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus rhamnosus* (as stated by the manufacturer). Rice milk was selected as the control because it is a non-fermented beverage containing no live microbial cultures. Neither beverage contained a meaningful amount of dietary fibre (kefir: 0 g per 250 ml serving; rice milk: 0.75 g per 250 ml serving) and neither was fortified with vitamin D. Participants were instructed as per the participant information leaflet to record any adverse effects.

Participants completed pre- and post-intervention questionnaires that contained validated scales for anxiety (Appendix A), stress (Appendix B) and GI health (Appendix C). Participants were also asked to record a food diary (Appendix D) pre- and post-intervention.

The primary outcome of interest was anxiety, with perceived stress and GI symptoms examined as secondary outcomes. The assessment of nutritional status, including omega-3, vitamin D and fibre intake, together with the relationship between GI health and psychological outcomes, was exploratory in nature and intended to inform hypotheses for future research.

Study population and sampling

Participants were recruited through non-probability convenience sampling from South East Technological University in Waterford. Individuals who contacted the research group were sent an eligibility criteria form (Appendix E) to determine suitable eligibility for participation. Participants who were eligible received a participant information leaflet (Appendix F). This leaflet highlighted the research background, goals and participant instruction. Prior to the trial taking place, an informed consent form (Appendix G) and GDPR form (Appendix H) were completed and signed by each participant.

Sixteen participants (six male and ten female) were selected using this sampling strategy. Recruitment was limited by the time available to complete the research

project and the cost of supplying the study drinks. A formal power calculation was not undertaken.

The inclusion criteria required participants to: (1) be aged 18–30 years old, (2) not be taking antibiotics nor have taken them in the previous three months, as antibiotics can alter gut microbiome, (Konstantinidis *et al.*, 2020) and (3) be willing to consume kefir for 14 consecutive days. The exclusion criteria required that selected participants must not: (4) be diagnosed with any gut related illnesses, (5) be diagnosed with severe psychiatric disorders and (6) be allergic to dairy or have a lactose intolerance.

Measurement tools and data collection procedures

Three-day food diary (Appendix F)

A food diary, including fluid intake, was completed by each participant before and after the intervention. The food diary was analysed observationally using dietary analysis software (Nutritics).

Gastrointestinal Gut Health Questionnaire (Appendix G)

This measurement tool assessed GI symptoms (Walter *et al.*, 2021: 1408). This tool provided an understanding of the participants' gut health.

The questionnaire adopted a scoring system represented by a scale from 0 to 100, in which participants rated their symptoms in accordance with the question. This indicated the severity of the gut health problem, with higher scores representing more severe conditions. The scales scores were added together without the participants' knowledge of their total. A score of over 210 from the first four questions indicated poor gut health. This tool was the primary measure of gut health and was administered pre- and post-intervention.

The Perceived Stress Scale (PSS) (Appendix H)

This measurement tool determined stress levels for each participant (Harris *et al.*, 2023:847–48). The tool consists of questions that relating to the participants' thoughts and feelings. Scores were derived from the participants' answers, where the range

represents the participants' feelings toward the question, which is answered from 0 (Never) to 4 (Very often). Some questions required reverse scoring as they were positively worded. The final scoring was calculated by summing the scores for each question. A score below 20 represented a low stress score; 20–30 represented a stressed score; finally, anything over 30 correlated to a very stressed score.

Generalised Anxiety Disorder (GAD-7) (Appendix I)

This measurement tool consists of seven questions to be answered by each participant. These questions all relate to common anxiety symptoms (Shevlin *et al.*, 2022), including symptoms such as nervousness, worry and irritability.

The scale ranges from 0 (Not at all) to 3 (Nearly every day). The scores from the seven questions were then added to determine the anxiety levels of each participant. Scores between 0–4 represented low anxiety, 4–8 was considered as mild anxiety, over 8 was considered high anxiety and a total score above 15 suggested severe anxiety levels. This tool was used pre- and post-intervention.

Data collection procedure

A Microsoft form was developed to include the GI symptom questionnaire, the PSS and the GAD-7 questionnaire. This form was sent out to each participant pre- and post-intervention. A food diary template was also distributed to each participant before and after the intervention. The completed forms were then sent back for analysis. This procedure allowed for accessible and efficient collection of data.

Data analysis

JASP was used as the statistical software for data analysis, with p-values < 0.05 considered statistically significant. Descriptive statistics were utilised to assess baseline data. A mean $\pm$ SD was used when displaying results.

To determine differences in participant characteristics between groups, an independent-samples t-test was used. Mann-Whitney U was used for data that was non-normally distributed. Welch's t-test was used where unequal variance was present. Paired

samples t-tests were conducted to determine the effectiveness of kefir in managing anxiety and stress by comparing pre- and post-intervention scores. To examine whether nutrient intake was associated with anxiety, stress or GI health, a Spearman’s rank correlation analysis was conducted. Lastly, to measure whether GI health was associated with anxiety or stress, a Spearman’s rank correlation analysis was also utilised. Correlation analyses were based on baseline data only.

Ethical approval

Ethical approval was received from the Ethics Committee of the School of Health Sciences at South East Technological University (SETU), Waterford.

Results

Participant characteristics and baseline data

Sixteen college students (age 21.19 ± 0.91 years; 6 male, 10 female) were randomly allocated, at a 1:1 ratio, to the kefir group (n = 8) or the control group (n = 8). On average, participants reported mild anxiety symptoms, low perceived stress and good GI health at baseline (Table 1). There were no statistically significant differences between groups in any baseline characteristic ($p > 0.05$).

Assumptions testing was conducted to determine the most appropriate statistical tests for each variable. Mann-Whitney U tests were employed in the case for non-normal distributions (GAD-7). Test of equality of variances highlighted unequal variances from stress scores ($p = .041$) and GI health ($p = 0.021$). Welch’s t-test was used for these respective variables.

Table 1: Baseline and descriptive characteristics with group comparisons between kefir and control groups

Measure	Total Sample (n = 16)	Intervention Group (n = 8)	Control Group (n = 8)	p-value
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Age	21.19 ± 0.91	21.5 ± 0.93	20.88 ± 0.84	0.197
GAD-7	8.63 ± 5.4	7.63 ± 4.07	9.62 ± 6.63	0.789
PSS	11.13 ± 3.38	10.75 ± 4.59	11.5 ± 1.77	0.676
GI health	91.06 ± 79.79	81.88 ± 38.54	100.25 ± 107.81	0.661

Values are presented in the table as mean ± standard deviation. p-values are taken from Mann-Whitney U tests where normality was not met and Welch's t-tests where unequal variance was indicated. No statistically significant differences were found between the intervention and control groups at baseline ($p > 0.05$).

Anxiety, stress and GI health outcomes following kefir intervention and control conditions

Paired sample t-tests revealed a significant reduction in GAD-7 scores in the kefir group ($t(7) = 2.65$, $p = 0.033$), with a large effect size ($d = 0.94$) (Table 2) (Figure 1). In contrast, no clinically significant change was observed in the kefir group's PSS scores ($t(7) = 0.66$, $p = 0.534$) with a smaller effect size ($d = 0.23$) (Figure 3). Although GI symptoms reduced, there was no significant difference observed ($t(7) = 2.17$, $p = 0.067$) (Table 2).

Significant reductions were found in the control group's GAD-7 scores ($t(7) = 3.97$, $p = 0.005$), with a large effect size ($d = 1.4$) (Figure 2). However, the assumption of normality was not met for these scores ($p = 0.002$). In contrast, the control group saw no significant change in the PSS scores ($t(7) = -0.489$, $p = 0.639$), with a negligible effect size ($d = -0.17$) (Figure 4). Finally, GI symptoms were reduced in the control group; however, no significant changes were highlighted ($t(7) = 1.37$, $p = 0.212$) (Table 2).

As shown in Table 2, independent-samples t-tests indicated no statistically significant difference between the kefir group and the control group in post-intervention reductions across anxiety ($p = 0.197$), stress ($p = 0.474$) or GI health symptoms ($p = 0.955$). All values were calculated post-intervention.

Table 2: Difference between GAD-7, PSS and GI health scores post-trial for intervention group and control condition

Domain	Group	Pre (Mean ± SD)	Post (Mean ± SD)	Mean difference	Effect size	p-value	
GAD-7 (Anxiety)	Kefir	7.63 ± 4.07	5.13 ± 4.67	2.5	0.94	0.033*	
	Control	9.63 ± 6.63	5.13 ± 5.49	4.6	1.4	0.005*	
						Between- group p-value	0.197
PSS (Stress)	Kefir	10.75 ± 4.59	10 ± 3.96	0.75	0.23	0.534	
	Control	11.50 ± 1.77	12.25 ± 3.88	-0.75	-0.17	0.639	
						Between- group p-value	0.474
GI Health	Kefir	81.88 ± 38.54	45.63 ± 32.67	43.34	0.767	0.067	
	Control	100.25 ± 107.81	65.75 ± 74.5	34.0	0.485	0.212	

						Between-group p-value	0.955
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*Mean difference pre- and post-mental health domains in the kefir and control group. Pre- and post-values are presented as $\pm$ standard deviation. The p-value indicates no statistically significant difference between groups. * indicates a statistically significant difference between pre- and post-values.*

Figure 1: Pre- and post-intervention GAD-7 scores in the kefir group (n = 8). Lines represent paired observations for each participant. Black markers denote the group mean $\pm$ 95% confidence interval

Figure 2: Pre- and post-intervention GAD-7 scores in the control group (n = 8). Lines represent paired observations for each participant, overlapping lines denote identical scores. Black markers denote the group mean $\pm$ 95% confidence interval

Figure 3: Pre- and post-intervention PSS scores in the kefir group (n = 8). Lines represent paired observations for each participant, overlapping lines denote identical scores. Black markers denote the group mean $\pm$ 95% confidence interval

Figure 4: Individual pre- and post-intervention PSS scores in the control group (n = 8). Lines represent paired observations for each participant. Black markers denote the group mean $\pm$ 95% confidence interval

Effect of GI health on anxiety and stress

Baseline GI symptom scores were not significantly correlated with anxiety ($\rho = 0.29$, $p = 0.271$) or stress ($\rho = 0.31$, $p = 0.239$) (Table 3).

Table 3: Correlation between GI health with anxiety and stress

Mental Health Domain	ρ	p-value	Significant Correlation?	Direction
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GAD-7 (Anxiety)	0.29	0.271	No	N/A
PSS (Stress)	0.31	0.239	No	N/A

Spearman's rho (ρ) is presented for the correlation between baseline GI health and anxiety and stress ($n = 16$). p -values represent statistically significant associations ($p < 0.05$).

Effect of nutrient intake (omega-3, vitamin D, fibre) on anxiety, stress and GI health

Mean values for each of omega-3, vitamin D and fibre were analysed across the sample ($n = 16$) (Table 4). There were no statistically significant correlations observed between nutrient intake with either mental health outcome (anxiety and stress) or GI health ($p > 0.05$) (Table 5).

Table 4: Mean daily intake of omega-3, vitamin D and fibre

Nutrient	Mean $\pm$ SD	Minimum	Maximum
Omega-3 (g)	1.57 $\pm$ 1.83	0.2	7
Vitamin D (μg)	4.54 $\pm$ 4.35	0.4	17.1
Fibre (g)	22.56 $\pm$ 8.66	12.1	43

Values are presented in the table as mean $\pm$ standard deviation

Table 5: Correlation between omega-3, vitamin D and fibre with anxiety, stress and gut health

Mental health domain	Nutrient	ρ	p-value	Significant correlation?	Direction
GAD-7 (Anxiety)	Omega-3	0.03	0.901	No	N/A
	Vitamin D	0.44	0.086	No	N/A
	Fibre	0.04	0.886	No	N/A
PSS (Stress)	Omega-3	0.16	0.543	No	N/A
	Vitamin D	0.10	0.715	No	N/A
	Fibre	-0.48	0.058	No	N/A
GI Health	Omega-3	0.09	0.731	No	N/A
	Vitamin D	0.02	0.950	No	N/A
	Fibre	0.07	0.807	No	N/A

Spearman's rho (ρ) is presented for correlations between baseline nutrient intake and anxiety, stress and GI health ($n = 16$). p-values represent statistically significant associations ($p < 0.05$).

Discussion

Summary of main research findings

The aim of this research was to examine the effectiveness of kefir yoghurt in managing anxiety, stress and GI health in a healthy population. This was accompanied by exploratory analyses of nutrient intake (omega-3, vitamin D and fibre) on the same variables as well as an examination of the relationship between GI health and psychological outcomes (anxiety and stress). A statistically significant reduction in GAD-7 scores across the kefir group and control group was observed in the trial's findings. However, there was no significant difference between groups post-intervention. This reduction may reflect placebo effects or natural changes in symptoms over time, and the small sample size may have limited the ability to detect a difference between groups.

In contrast, changes in PSS scores were not statistically significant, which may limit kefir's broader applicability as a therapeutic intervention in managing stress. Additionally, the absence of a statistically significant difference in GI health scores post-intervention in the kefir group weakens the claim that probiotics improve anxiety or stress through improving GI health. Moreover, no significant correlation emerged between baseline GI symptoms and anxiety or stress, which is inconsistent with previous research in this area (Bloom *et al.*, 2021; Cryan *et al.*, 2019:1877).

Similarly, no significant correlations were revealed between nutrient intake (omega-3, vitamin D and fibre) and levels of anxiety, stress or GI health. This discussion will expand on these findings by addressing the study's limitations and offering direction and guidance for future research.

Descriptive characteristics and baseline data

Baseline data offered a strong foundation for evaluating the effectiveness of the intervention. There were no statistically significant differences observed between the intervention and the control group across age, anxiety, stress or GI health, which confirmed comparability. This finding suggests that randomisation was successful, which boosts the internal validity of the randomised control trial. Baseline scores also indicated mild anxiety, low perceived stress and good GI health, which provide important context in determining the effectiveness of the intervention.

Effectiveness of kefir in managing anxiety and stress

Evidence from this study highlighted significant reductions in GAD-7 scores in both the intervention and control groups. This suggests that kefir alone did not influence anxiety outcomes in the sample. These findings may relate to the potential of a placebo response taking place, which is often a challenge within the area of nutritional psychiatry. Similar conclusions were reported by Moschinis *et al.* (2024), where both the intervention group and the placebo group showed similar reductions in anxiety after 6 weeks and 12 weeks of probiotic consumption, with no significant reduction observed between groups. This highlights the premise that psychological outcomes may be influenced by external factors rather than the active component in isolation.

Additionally, this intervention focused on a non-psychiatric population. This study design was similar to Tran *et al.* (2019: 271), who implemented a 28-day probiotic intervention in healthy young adults. Their study reported no significant between-group differences, which was also a determinant factor in the present study. Tran *et al.* (2019: 274) also determined that a ceiling effect may have influenced their findings, which may have similarly skewed results for this study. This observation suggests that detecting the effectiveness of the intervention may prove challenging when participants do not present with elevated symptoms of anxiety. This interpretation is supported by a meta-analysis, which found that the effects of probiotics on anxiety and depression were negligible in non-clinical samples compared with clinical samples (Liu *et al.*, 2019: 13).

In contrast, there were no significant changes in either group for stress. This suggests that kefir did not provide any meaningful effects for improvements in perceived stress. Other research has contradicted these findings, inferring that probiotic intake may influence stress outcomes through cortisol regulation or through anti-inflammatory avenues (Jain *et al.*, 2024). The two-week time intervention period may have been insufficient to elicit notable changes in chronic stress perception. Studies have suggested that probiotic intake may only elicit reductions in cortisol and pro-inflammatory markers after 12 weeks (Lew *et al.*, 2019: 2053). Significant changes in stress scores from this study are likely to have required an longer intervention period to allow these biological pathways to materialise.

Other research in this area has also mentioned that stress-based probiotic interventions may only yield significant effects in populations exposed to high stress (Papalini *et al.*, 2018), in contrast to the present study, where stress levels were only moderate.

Additionally, kefir intake did not significantly reduce symptoms associated with GI discomfort. This further weakens the hypotheses that kefir improves gut health, which indirectly improves either stress or anxiety.

These findings underscore the complexity of evaluating probiotic interventions for psychological well-being, where external factors and limited evidence continue to obscure their true significance.

GI health's role in managing anxiety and stress

Findings from this study demonstrated no significant correlation between baseline GI health and anxiety. These outcomes are inconsistent with previous research, which supports the notion that GI health influences anxiety outcomes (Wang *et al.*, 2023: 4926) and suggests that individuals with high levels of anxiety also exhibit poorer GI health (Tayama *et al.*, 2017: 1). The small sample size and generally good GI health of participants may have limited the ability to detect this association.

Similarly, this research found no correlation between GI health and stress. This relationship is perhaps slightly ambiguous, reflecting the complex nature of stress. While anxiety is at times easier to identify and usually follows a more physiologically distinct pattern, perceived stress can be contextual and often difficult to define clearly (Nater, 2021: 1265). Future research should focus on the multidimensional assessments of perceived stress, which is proportionate to its contingent nature.

The conclusions from this study in relation to GI health, anxiety and stress highlight the need for future investigation with the necessary study design improvements.

The effects of omega-3, vitamin D and fibre intake on anxiety, stress and gastrointestinal health

In the exploratory analyses, this study found no significant correlation between nutritional intake and anxiety or stress. These findings were inconsistent with previous

research, which has highlighted the roles of omega-3, vitamin D and fibre in producing certain positive effects on related psychological outcomes and GI health (Cheng *et al.*, 2020: 553; Esmailzadeh *et al.*, 2021; Kumarasivam *et al.*, 2022). The notably low intake of omega-3, vitamin D and fibre indicated that several participants reached sub-clinical levels for each of the chosen nutrients. This may provide an initial reasoning for the absence of significant correlation between these variables.

Additionally, no significant correlations were found when assessing the relationship between these nutrients and GI health. These findings are inconsistent with previous studies, which have demonstrated the benefits of omega-3, vitamin D and fibre in improving GI health (Fu *et al.*, 2021: 4; Singh *et al.*, 2020; So *et al.*, 2018: 969). Assessing omega-3's ability to improve GI health is often complicated by factors such as dose, individual metabolism and variability in gut microbiota response to the nutrient (Fu *et al.*, 2021: 6; Zhu *et al.*, 2021: 1), none of which were measured in this study. Additionally, difficulty in relating vitamin D to GI health presents challenges in smaller sample sizes (Bashir *et al.*, 2015: 1487), which was apparent in the current study. Lastly, linking fibre to improvements in GI health may also prove difficult in studies like this one. Fibre's physicochemical properties can vary based on origin and processing of the nutrient, which can lead to changes in bioavailability and contribute to unreliable findings in data (Gill *et al.*, 2020: 101).

As this study revealed no significant correlation between these nutrient intake and GI health, the complexity of confounding factors such as sufficient intake, individual variability and nutrient uptake provides a potential reasoning for this absence of linear association.

Limitations

Generalisability was limited due to the sample being constrained to college students. Previous studies have suggested that physiological outcomes and gut microbiota composition can be affected by age, gender and lifestyle-related variation (Cryan *et al.*, 2019: 1877; Filmonova *et al.*, 2024). This may affect applicability when linking findings to a general population.

In addition, a short-term intervention period of 14 days may have proved insufficient to produce any significant differences relating to anxiety, stress or GI health, potentially reflecting transient outcomes rather than the development of systemic changes. Several studies have highlighted that changes in underlying mechanisms relating to anxiety, stress and gut microbiota composition may require sufficient time to develop (Lew *et al.*, 2019: 2053; Ma *et al.*, 2021: 1).

Limitations extended to the sample size (n=16), which reduced the reliability of the data and increased the possibility of a **Type II error**, which may have prevented the true effectiveness of kefir from being detected.

Given that the study relied on self-reported data for all outcome variables (anxiety, stress and GI health), the results may also be subject to certain biases (Gorber and Tremblay, 2016: 369). This reliance could have led to inaccuracies in the data due to the potential of under- or over-reporting for certain symptoms (Gorber and Tremblay, 2016: 371).

This study also did not account for potential confounding factors such as physical activity, stress exposures or sleep, all of which may have hindered the independent effects of kefir and nutrient intake on psychological outcomes (Bozdoğan *et al.*, 2024).

Future recommendations

Future research should incorporate a longer trial period. Probiotics can take a minimum of 8 weeks to elicit measurable psychological effects (Ma *et al.*, 2021:1). Extending the duration of the intervention would provide a suitable environment for a more accurate representation of probiotics' influence on mental well-being.

To improve statistical power and reduce the chance of a Type II error, a larger sample size should be used in future research. Trials that are at risk of this error often lack sensitivity to identify significant effects (Mukti, 2025: 127).

To increase the robustness of the methodology, future studies should focus on objective adherence tracking such as blood and stool sampling. Adding this biological verification would help to provide non-biased and accurate measures of data

(Nogueira-Perez *et al.*, 2025). Lastly, enabling a measure to control for confounding factors such as habitual diet, stress exposure or sleep would help establish a clearer understanding of the isolated effects of probiotics and nutrient intake on psychological outcomes (Bozdoğan *et al.*, 2024).

Application of findings

Given the absence of a between-group difference, these findings do not support recommending kefir for the management of anxiety or stress. However, as an accessible and affordable product, kefir may warrant further investigation in larger scale trials.

Conclusion

Anxiety scores decreased in both the kefir and control groups, with no significant difference between groups. There were also no significant effects in reducing stress-related outcomes or improving gastrointestinal health. While nutrient intake (omega-3, vitamin D and fibre) was recorded, the findings revealed no significant associations between nutrient intake and anxiety, stress or GI health. These findings provide preliminary data on kefir use in a healthy, non-clinical population, which remains under-researched. This study did not find a between-group difference indicating a meaningful impact of kefir on anxiety or stress symptoms over the control condition, underscoring the importance of conducting extended-duration trials with larger sample sizes in future probiotic research.

Acknowledgements

I would like to express my sincere gratitude to my incredible supervisors, Bruce Wardrop and Dr Lorna Doyle, for their unwavering support, insightful guidance and shared passion for research. Their wisdom, encouragement and dedication have been invaluable throughout this journey.

List of figures

Figure 1: Pre- and post-intervention GAD-7 scores in the kefir group (n = 8). Lines represent paired observations for each participant. Black markers denote the group mean $\pm$ 95% confidence interval

Figure 2: Pre- and post-intervention GAD-7 scores in the control group (n = 8). Lines represent paired observations for each participant, overlapping lines denote identical scores. Black markers denote the group mean $\pm$ 95% confidence interval

Figure 3: Pre- and post-intervention PSS scores in the kefir group (n = 8). Lines represent paired observations for each participant, overlapping lines denote identical scores. Black markers denote the group mean $\pm$ 95% confidence interval

Figure 4: Individual pre- and post-intervention PSS scores in the control group (n = 8). Lines represent paired observations for each participant. Black markers denote the group mean $\pm$ 95% confidence interval

List of tables

Table 1: Baseline and descriptive characteristics with group comparisons between kefir and control groups

Table 2: Difference between GAD-7, PSS and GI health scores post-trial for intervention group and control condition

Table 3: Correlation between GI health with anxiety and stress

Table 4: Mean daily intake of omega-3, vitamin D and fibre

Table 5: Correlation between omega-3, vitamin D and fibre with anxiety, stress and gut health

Appendices

Appendix A

Generalised Anxiety Disorder 7 Self-Report Anxiety Test

This questionnaire called the GAD-7 screening tool can help you find out if you might have an anxiety disorder. It calculates how many common symptoms you have and based on your answers suggests where you might be on a scale, from mild to severe anxiety. Your answers should be based off the last 5 days.

	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it's hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid, as if something awful might happen	0	1	2	3
Add up your results for each column				
Total score (add column totals together)				

Appendix B

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

The questions in this scale ask you about your feelings and thoughts in the last week. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

Name _____ Date _____ Age _____

Gender (Circle): M F Other _____

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the week, how often have you been upset because of something that happened unexpectedly?..... 0 1 2 3 4

2. In the last week, how often have you felt that you were unable to control the important things in your life?..... 0 1 2 3 4

3. In the last week, how often have you felt nervous and “stressed”?..... 0 1 2 3 4

4. In the last week, how often have you felt confident about your ability to handle your personal problems?..... 0 1 2 3 4

5. In the last week, how often have you felt that things were going your way?..... 0 1 2 3 4

6. In the last month, how often have you found that you could not cope with all the things that you had to do?..... 0 1 2 3 4

7. In the last week, how often have you been able to control irritations in your life?..... 0 1 2 3 4

The Short Health Scale for Gastrointestinal Symptoms

The Short Health Scale for GI symptoms (SHS-GI) is a simple questionnaire to measure the impact of GI inconvenience and symptoms on quality of life.

1. Do you have any symptoms/inconvenience from gastrointestinal tract?

No symptoms = 0 Very severe symptoms = 100

Your answer: _____

1. Are your daily life activities interfered by gastrointestinal symptoms/inconvenience?

Not at all = 0 Interferes to a very high degree = 100

Your answer: _____

1. Do you have worry caused by gastrintestinal symptoms/inconvenience?

No worry = 0 Constant worry = 100

Your answer: _____

1. What is your general sense of wellbeing?

Very well = 0 Dreadful = 100

Your answer: _____

Appendix D

Food Diary

Food	Amount/Portion Size	
(Include all foods, drinks and supplements)	(for example ½ cup, 3 oz)	

Breakfast or 1 st meal Time:			
Snack Time:			
Lunch or 2 nd meal Time:			
Snack Time:			
Dinner or 3rd meal Time:			
Snack Time:			

Appendix E

Eligibility Criteria Form This form is set out to acquire appropriate individuals to be able to participate in the study
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<p>1: Full Name</p> <p>_____</p> <p>2. Date of Birth</p> <p>_____</p> <p>3. Contact Information</p> <p>_____</p> <p>4. Are you a full-time student enrolled in South East Technological University?</p> <p>Yes ___ No ___</p> <p>5. Are you over 18?</p> <p>Yes ___ No ___</p> <p>6. Are you vegan?</p> <p>Yes___ No _____</p>	<p>7. Are you able to commit to a 2 week study where you will be asked to follow a strict regime and protocol?</p> <p>Yes___ No _____</p> <p>8. Are you lactose intolerant?</p> <p>Yes___ No _____</p> <p>9. Are you vegan?</p> <p>Yes___ No _____</p> <p>10. Do you take probiotic supplements?</p> <p>Yes___ No _____</p> <p>11. Are you currently taking psychiatric medication?</p> <p>Yes___ No _____</p>
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Appendix F

Participant Information Leaflet

Title: An investigation into the effectiveness of Kefir in Managing Stress and Anxiety in College Students. A Randomised Controlled Trial with Assessment of Nutritional Status.

Introduction to the leaflet:

This leaflet stands to provide you, the participant, with all the information based on the study being conducted. This study seeks to investigate the effects of probiotics on mental health outcomes amongst a student population. Read this information carefully and do not hesitate to bring forward any queries or concerns. We hope you are as excited as we are to get started!

Background:

There has been emerging evidence to suggest a link between probiotics contributing to a healthy gut to improve mental health. Unfortunately, many studies provided have been performed on animals. In addition to this many studies have found an indirect link between probiotics and mental health parameters like stress and anxiety. This is where you come in. We want to bridge the gap that exists in the context of probiotics, mental health and gut health. By using participants who are human to find a direct link between the probiotics and mental health.

Our goal:

We want to remodel the way mental health is approached. Completing this study can uphold the ability for practitioners to have options when it comes to helping patients with mental health issues. With the countless medications available for mental health, why not provide one which has minimal side effects, with a more holistic approach rather than focusing on the brain? Our goal is to help the people who feel helpless by providing them with another option, one they've never heard of.

What do you have to do?

You will be required to answer a selection of questionnaires to gather baseline mental health and gut health scores. You will then be required to complete these questionnaires again when the study has reached its conclusion. During the 2 week research phase you will be asked to adhere to strict protocols surrounding the study. This is to ensure accuracy and efficacy when conducting results. The protocols are as follows:

1. You will be given kefir which contains probiotics. This is to be taken daily.
2. You won't take any additional probiotic product or supplement during the 2 weeks.
3. You will inform the research team if you have been prescribed antibiotics.
4. You will reach out to the research team if you experience any negative side effects.
5. You must keep the details of the study private.
6. If you wish to withdraw yourself from the study, you must contact us.

Risks and Benefits:

There are minimal risks associated with this study. This study may provide an opposite reaction for participants. Benefits may arise in the form of improved gut health and mental health. Please adhere to protocol 4 if any risks do arise.

Confidentiality:

Your data will be kept completely private confidential and will be aligned with the General Data Protection Regulation (GDPR).

I have understood and acknowledged the participant information leaflet.

Questions or queries:

Participant Signature

Date.....

Appendix G

Participant Informed Consent Form

I have read and fully understood the participant information leaflet.	Yes	No
I will follow the protocols set out in the participant information leaflet.	Yes	No
I am fully aware of the potential risks associated with this research.	Yes	No
I understand that I am a volunteer and can withdraw my participation at any stage, without providing a reason.	Yes	No
I give informed consent to take part in this study.	Yes	No

Participant signature:..... Date:.....

Appendix H

GDPR Consent Form

Title: An Investigation into Impact of Probiotics present in Kefir Yoghurts on Anxiety, Stress, Sleep Patterns, Gastrointestinal Symptoms and Nutrient Intake in College Students

Data Collection:

During this study we will collect data that will include but is not limited to your age, health, consent, mental health and gut health scores. This data is of upmost importance to determine if probiotics do impact mental health outcomes in college students.

Your Data Protection Guarantee:

Your data will be stored in a secure location with password only access. Any personal identifiers will be separated from the research data. Your data will be held for the duration of the study plus 2 weeks after the study grade has been awarded by SETU. This will allow time for the research team to evaluate their findings.

Your Rights for Data:

Under GDPR requirements you have the right to access or request deletion of your data at any time

I understand the information around my data. I understand my rights under the GDPR requirements and give full consent for my data to be accessed and stored.

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

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Glossary

Gut-brain axis: An interconnected communication pathway that links the gut microbiota with the central nervous system.

Microbiota: Refers to a community of microorganisms including bacteria, fungi and archaea. Microbiota clusters inhabit specific environments in or on the human body, such as the gastrointestinal tract, skin, mouth, reproductive tract and eyes. These microbial communities offer roles in digestion, immune function and overall health.

Cortisol: A steroid hormone produced by the adrenal glands. It is commonly referred to as the ‘stress hormone’ due to its involvement in the body’s response to stress.

Saccharomyces: A genus of yeast made up of species with complex cells. Saccharomyces yeast facilitates certain roles in food, pharmaceuticals and vaccine production.

Leuconostoc: Embodies a range of species that often appear in food where they play several roles which can range from ripening to spoiling.

Bifidobacterium: Positive bacteria that reside in the gut and encompass an ability to support gastrointestinal conditions, mental health, respiratory function and skeletal and muscular health.

Lactobacillus: Bacteria of the genus *Lactobacillus* are inhabitants of human mucous membranes which are involved in maintaining health and reducing likelihood of pathological conditions.

IL-1 β : Enables sufficient anti-inflammatory responses to negate infection and disease, while simultaneously if not controlled suitably can trigger inflammation and contribute to disease.

Alpha diversity: The variety of microbial species within a single individual’s gut, based on how many species are present and how evenly they are spread.

Beta diversity: The difference in microbial composition between individuals or samples.

Gut microbiome diversity: The range and balance of different microorganisms living in the gut. Greater diversity is generally linked to better gut and overall health.

Probiotic: Live microorganisms that provide a health benefit to the host when consumed in sufficient amounts.

Firmicutes-to-Bacteroidetes ratio: The relative abundance of two major groups of gut bacteria, Firmicutes and Bacteroidetes. Shifts in this ratio have been linked to changes in gut health and inflammation.

Type II error: Failing to detect a real effect, wrongly concluding there is no difference when one actually exists. It is more likely in studies with small sample sizes.

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