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Randomised controlled trial of the effects of kefir on behaviour, sleep and the microbiome in children with ADHD: a study protocol

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ABSTRACT

Introduction Current interventions for children with attention-deficit/hyperactivity disorder (ADHD) are primarily medication, behavioural therapy and parent training. However, research suggests dietary manipulations may provide therapeutic benefit for some. There is accumulating evidence that the gut microbiome may be atypical in ADHD, and therefore, manipulating gut bacteria in such individuals may help alleviate some of the symptoms of this condition. The aim of this study is to explore the effects of supplementation with kefir (a fermented dairy drink) on ADHD symptomatology, sleep, attention and the gut microbiome in children diagnosed with ADHD.

Methods and analysis A 6-week randomised, double-blind, placebo-controlled trial in 70 children aged 8–13 years diagnosed with ADHD. Participants will be recruited throughout the UK, through support groups, community groups, schools, social media and word of mouth. Children will be randomised to consume daily either dairy kefir or a placebo dairy drink for 6 weeks. The primary outcome, ADHD symptomatology, will be measured by The Strengths and Weakness of ADHD-symptoms and Normal-behaviour scale. Secondary outcomes will include gut microbiota composition (using shotgun metagenomic microbiome sequencing), gut symptomatology (The Gastrointestinal Severity Index questionnaire), sleep (using 7-day actigraphy recordings, The Child's Sleep Habits Questionnaire and Sleep Self Report questionnaire), inattention and impulsivity (with a computerised Go/NoGo test). Assessments will be conducted prior to the intervention and at the end of the intervention. Interaction between time (preintervention/postintervention) and group (probiotic/placebo) is to be analysed using a Mixed Model Analysis of Variances.

Ethics and dissemination Ethical approval for the study was granted by St Mary's University Ethics Committee. Results will be disseminated through peer-reviewed publications, presentations to the scientific community and support groups.

Trial registration number NCT05155696.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The trial is randomised and blinded, which is critical to minimise expectation bias.
⇒ It employs a variety of objective and subjective outcome measures, providing a comprehensive assessment of potential impacts.
⇒ It explores a potential mechanism (changes in gut microbiota) by which diet may exert an effect on behaviour.
⇒ While the design is adequately powered, results may require replication in a larger sample to increase confidence in them.
⇒ The study population will be self-selecting, comprising families who are receptive to dietary interventions. This may be a potential threat to external validity, as findings may not extrapolate to the wider aattention-deficit/hyperactivity disorder population.

INTRODUCTION

Background and rationale

Aattention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder with a global community prevalence, in children, of around 5%.[1] ADHD is defined by its behavioural symptoms of impulsivity, hyperactivity and inattention.[2] In addition to these core symptoms, children with ADHD often suffer from co-occurring mental health difficulties,[3] have poorer educational outcomes than their non-affected peers[4] and are more likely to have sleep difficulties.[5 6] Estimates suggest that between 50% and 85% of children diagnosed with ADHD may experience some kind of sleep difficulties[7 8] and these difficulties persist across the lifespan.[9] ADHD has a significant impact on individuals, families and societies, with a marked impact on quality of life[10] and significant economic costs both in the USA and Europe.[11 12]

Therapeutic interventions typically implemented by health authorities are targeted at modifying the underlying neurochemistry of ADHD by use of medication, such as methylphenidate,[13] or modifying behaviour with the use of behaviour therapy or parent training.[14] However, over the past few decades, there has been emerging interest in the use of dietary therapies to help manage symptoms of ADHD.

Research in this area is encouraging, although studies are often hampered by small sample sizes and inconsistent findings. The exclusion of artificial food colourings[15 16] and supplementation with essential fatty acids[17–19] have been found to improve ADHD symptomatology in some studies. However, systematic reviews suggest that results are inconclusive and that more research is needed.[20] One of the most effective dietary interventions in children with ADHD is the few foods diet, which excludes foods most likely to provoke sensitivities, requiring the child to eat just a small number of whole-food items (such as lamb, chicken, potatoes, rice, banana, apple and Brassica).[21] Such a diet would be difficult to maintain for a duration of time outside of a clinical trial. We have proposed elsewhere[22] that one potential mechanism that could mediate the success of these diets is the potential they have to alter gut microbiota composition. Emerging research is beginning to suggest that the composition of the microbiota may be atypical in individuals with ADHD[23–25] and it has been reported that children with ADHD may have increased incidence of gastrointestinal symptoms.[26] Dietary interventions that are specifically designed to target the gut microbiota may help induce improvements in ADHD symptomatology.

We conducted a small feasibility study on a diet designed to impact the composition of gut microbiota on nine children with ADHD. The diet (which included increased consumption of plants; a 12-hour overnight fast; daily kefir consumption; consumption of a protein-rich breakfast and reduction in sugar intake) was well tolerated and rated as highly acceptable by the parents.[22] However, recruitment for the study was challenging, which led us to conclude that recruitment for a large-scale randomised controlled trial (RCT) would be problematic. Thus, we propose a simplified dietary intervention to use in the current RCT, in the hope of enhancing recruitment. The aspect of the previous dietary protocol with the highest level of adherence was daily consumption of kefir (at 97.6% compliance). This aspect directly introduced beneficial bacteria to the gut and is also the easiest aspect of the diet to match with a control condition and to achieve blinding in participants and researchers.

Kefir is a fermented probiotic drink which is created through the 'symbiotic fermentation of milk by lactic acid bacteria and yeasts contained within an exopolysaccharide and protein complex called a kefir grain' (Bourrie BCT,[27],p. 1). The fermented product contains a variety of probiotic bacteria (often including species of the former genus *Lactobacillus, Lactococcus, Streptococcus* and *Leuconostoc*) and yeast species (commonly including *Saccharomyces, Kluyveromyces* and *Candida*).[27 28] It has been demonstrated that the consumption of kefir can positively alter the composition of the gut microbiome, in both animal models[29 30] and human studies.[31 32] Preliminary evidence, from small-scale animal models, suggests that supplementation with kefir may be linked to behavioural effects such as less fatigue[33] and changes in reward-seeking and repetitive behaviour.[30] The mechanisms by which kefir influences the microbiome are still being explored, but it is plausible that it exerts an effect in at least three different ways. First, the administration of kefir directly introduces beneficial bacteria and yeasts into the gastrointestinal tract. Second, in vitro studies have revealed that kefir has antimicrobial properties against various strains of pathogenic bacteria and yeasts such as *Escherichia coli*,[34] *Salmonella typhi* and *Candida albicans*.[35] Therefore, it is possible that it enhances the microbiome through the process of reducing the prevalence of less beneficial bacteria and yeasts. Finally, kefir may exert an effect by promoting the growth of beneficial microbes already present in the gut.[27]

The association between diet and ADHD, first postulated by Feingold,[36] associates a non-optimal diet with ADHD,[37] with exacerbation of ADHD symptoms when certain foods are introduced.[20 38] Several theories have been proposed to explain this association, such as metabolic and/or mitochondrial dysfunction, immune mediated hypersensitivity, gastrointestinal inflammation and/or gut sensitivity, abnormality of fatty acid metabolism and amino acid deficiency.[39] Of interest to this study is the theory that a compromised gut microbiome may lead to the proliferation of opportunistic, pathogenic bacteria, viruses and fungi in the digestive tract, which risk being absorbed in the blood stream and carried to the brain,[40 41] which may then impact on behaviour.

Although there is no previous research exploring the effects of kefir in children with ADHD, we believe that daily consumption of this drink should be feasible in this population. We propose that kefir consumption may enhance composition of the gut microbiota in children with ADHD and could lead to a reduction in ADHD symptoms and improvements in sleep.

This paper presents the protocol for our RCT of the effects of kefir on behaviour, sleep and the microbiome in children with ADHD. The primary aim of this study is to assess the effects of kefir on ADHD behaviour. Secondary aims are to assess the effects of kefir on gut microbiota composition, gut symptomatology, sleep, inattention and impulsivity. We hypothesise that supplementation with kefir will be superior to supplementation with a placebo drink at inducing improvements in ADHD symptoms.

METHODS

Study design

This is a 6-week, parallel group, double-blind, randomised controlled trial of the supplementation of daily kefir versus a placebo dairy drink in children with ADHD. This trial was registered with ClinicalTrials.gov (Identifier: NCT05155696). It has been developed and reported in accordance with the Standard Protocol Items: Recommendations for Interventional Trials guidelines.[42] Primary and secondary outcomes will be measured through assessments conducted prior to commencement of the intervention (at baseline) and at completion (week 6). The

study commenced in January 2022 and completed data collection in September 2023.

Study location

The study is based in the UK with participants taking part within their own home.

Randomisation

To avoid the imbalance that can be inherent in relatively small trials, a baseline adaptive randomisation procedure is used. This sequentially assigns new subjects to a treatment group based on the covariate values for this subject while also taking into account all previously randomised subjects. Covariates for this study are age, sex and medication status. This way, a balance in the covariates among the treatment groups is achieved.[43 44] Simple randomisation where baseline covariates are used to take account of their possible effects on the trial outcome may result in the imbalance of these covariates among the treatment groups. This trial will implement an algorithm which uses baseline adaptive randomisation[45] in Excel.

Participants

Inclusion and exclusion criteria

Children are eligible to take part in the study if they have received a diagnosis of ADHD according to the Diagnostic and Statistical Manual of Mental Disorders version four or five (DSM-IV or DSM-V) criteria by a specialist qualified healthcare professional and are aged between 8 years and 13 years at onset of study. This is verified by the screening questionnaire. Age limits were set based on the questionnaire measures used to measure the outcomes of sleep and ADHD symptomatology. Males and females, children with co-occurring diagnoses and those with food allergies or sensitivities are eligible to take part in the study. Since comorbidity is very common for children with ADHD,[46] including such children allows us to recruit a representative sample.

Participants are not eligible to take part if they: (1) are currently undergoing a course of behavioural therapy; (2) have a milk allergy or lactose intolerance; (3) report use of antibiotics, probiotics, antifungals or steroids in the past 4weeks; (4) have a diagnosis of a gastrointestinal disorder, for example, inflammatory bowel disease or coeliac disease and (5) have a diagnosis of an autoimmune disease or compromised immunity. Originally, we planned to exclude those currently taking ADHD medication. However, following difficulties in recruitment, we removed this exclusion criteria and decided to include children on stable medication (ClinicalTrials.gov version 2, 30 Nov 2022) as recommended in recent research.[47] Since ADHD medication may influence both behaviour and the gastrointestinal system in these children, we will take this up as a factor in the analysis of the results.

Sample recruitment

Participants will be recruited through the community, using convenience sampling. Information about the study will be distributed via email, flyers and social media advertising facilitated by ADHD support groups, local community groups and schools based in the UK. Participants will be informed that the study is a randomised controlled trial (RCT) looking at the effects of a probiotic drink in children with ADHD. Parents of prospective participants are asked to contact the trial manager who then provides further information either via email and/or telephone and parents and children have the opportunity to ask any questions. Parents then complete a short screening questionnaire, administered via Jisc Online Surveys (Jisc, Bristol, UK), to determine whether their child is eligible to participate.

Enrolment into the study will occur on a rolling basis, ceasing when the target number of participants has been met or time/resource-constraints force cessation of the study. Participants will be randomised into a treatment or placebo group using block randomisation stratified for age, sex and medication status. Group allocation is decided by an independent researcher at St Mary's University who notifies the drinks distribution company. The primary researchers, participants and their families are blinded to the allocation.

Sample size calculation

We aim to recruit 35 participants per group, allowing for a 20% drop-out rate. A sample size of 27 per group was estimated using software G*Power V.3.1, based on analysis of variance (ANOVA) repeated measure, within-between interaction using an alpha level of 0.05, a power (1–ß) of 0.95 with an effect size of f=0.25 between measures. During the setup of the study, we recruited a statistician to the team and have adopted a more effective method of analysing the data, which compares the end point values between the groups, while taking into account the preintervention variability in the scores (thus the effect size for this would be d=0.5, with d=2×f). When the analysis is run, the SD will possibly be reduced by applying the correction for differences in a priori scores—this will make a difference to the power to detect an effect and also result in a higher Cohen's f and d. The sample size calculation is justified in the following ways: (1) In our previous single-group feasibility study,[22] the change in Parent report ADHD T-Score was approximately d=0.36. While this value may not directly represent the difference in changes between a treated and an untreated group, it provides valuable insight into the magnitude of change in a primary outcome measure and serves as a starting point for our justification. (2) We have adopted a new primary outcome measure (the Strengths and Weakness of ADHD-symptoms and Normal, SWAN scale) to use in this study, as opposed to the Conners Clinical Index (which was used in the feasibility study). Ratings on the SWAN cover a broader range of attention skills, from difficulties with attention and hyperactivity through to positive attention skills[48] and thus may afford us greater sensitivity to changes following intervention as it is more sensitive to changes at both ends of the spectrum.[49] (3) Effect sizes in dietary interventions are often small-moderate.[19 50] It is

our view that a medium effect size would be most appropriate to determine whether the intervention elicits meaningful change and has practical importance for clinical intervention and patient quality of life beyond statistical significance. (4) As a precautionary measure, a statistical power of 0.95 was selected, to account for scenarios where the drop-out rate surpasses initial estimate or the effect size proves to be smaller than anticipated.

Patient and public involvement

Patients and families were first involved in the research at the conception, development, execution and evaluation of the pilot study, preceding this trial, which assessed a broader microbiome targeted dietary intervention in ADHD.[22] In particular, families informed the decision to simplify the dietary intervention to using kefir alone and also to use online questionnaire and experimental outcome measures. Talks and webinars will be offered by the research team to ADHD support groups throughout the recruitment process and after study completion, to disseminate findings.

Intervention

Participants will be asked to consume 125 mL of study drink per day for 6 weeks. Both intervention and control drinks are supplied in plain packaging and delivered chilled using the same courier company. During the intervention, participants will be asked to maintain their usual diet and daily routine. Drinks are distributed to participants at the beginning of the study and at the midpoint to allow for adequate storage space in the fridge. Participants will be asked to consume drinks every day for the duration of the study. The drink can be taken on its own, with food, or combined into a smoothie. Recipe ideas for how to combine the drink into smoothie drinks or bowls are provided.

Intervention group

Participants allocated to the intervention arm will consume kefir. Nourish Kefir is supplying the organic cow's milk kefir for the study. The kefir is estimated to contain approximately 50 billion live micro-organisms per 125 mL serving. Species of micro-organisms vary due to fermentation but typically include species from the following bacterial genera: *Leuconostoc*, *Lactococcus*, *Bifidobacterium* and the former *Lactobacillus*, as well as *Saccharomyces* yeast species, and the exopolysaccharide kefiran.

Control group

Participants in the placebo arm will consume ultra heat treated (UHT) cow's milk. It contains none of the live microorganisms that are present in kefir, and additionally contains no extra ingredients that could potentially cause either negative or positive impacts—it is safe for consumption.

Adverse event recording and management

Potential adverse effects of consuming kefir include gastrointestinal symptoms, such as bloating and flatulence.[51] Adverse events will be recorded according to the Common Terminology Criteria for Adverse Events[52] and European Commission guidelines[53] throughout the duration of the study. Parents are asked to report any adverse events immediately to the research team. In the event of significant gastrointestinal distress, participants will be asked leave the trial and asked to consult their general practitioner if problems persist. Parents/caregivers will be able to contact the research team for specific advice and guidance regarding any adverse events. Adjustments may be made on an individual basis as necessary with support from the research team. Participants will be made aware that they are free to withdraw from the study at any time and will be advised to do so if adverse effects are troublesome. If adverse events were particularly problematic, a researcher independent of the study team could unblind the group allocation for this participant and communicate this directly. The rest of the participants and all researchers would remain blind to group allocation.

Adherence

Adherence is monitored by parent report of missed drinks. Families are provided with a chart to stick on their fridge to keep a record of daily adherence. This is returned to the research team at the end of the study.

Participant timeline

The schedule and time commitment for each group is shown in figure 1.

Outcome measures

All outcome measures have been developed and/or used with children between the ages of 8–13 years. Measures were selected based on their frequency of use within paediatric ADHD samples and their psychometric properties.

Primary outcome measure

The primary outcome measure is ADHD symptomology, as measured by The SWAN-behaviour scale.[54] The SWAN scale is an 18-item questionnaire, validated for use in children 6–18 years[55 56] and is completed online by parents and teachers. They are asked to rate the child by comparing them to other children of the same age group. Each item is responded to on a 7-point scale ranging from far below average (3) to far above average (−3), with 0 being average. The scores are summed and divided by the number of items, to express the summary score as the average rating per item (range −3 to+3, with higher scores indicating more severe ADHD symptoms). Nine items are averaged to compute an inattention subscale and nine items are averaged to compute a hyperactivity/impulsivity subscale. The scale has been reported to have good internal consistency; acceptable longitudinal stability[49]; and good discriminant validity.[56]

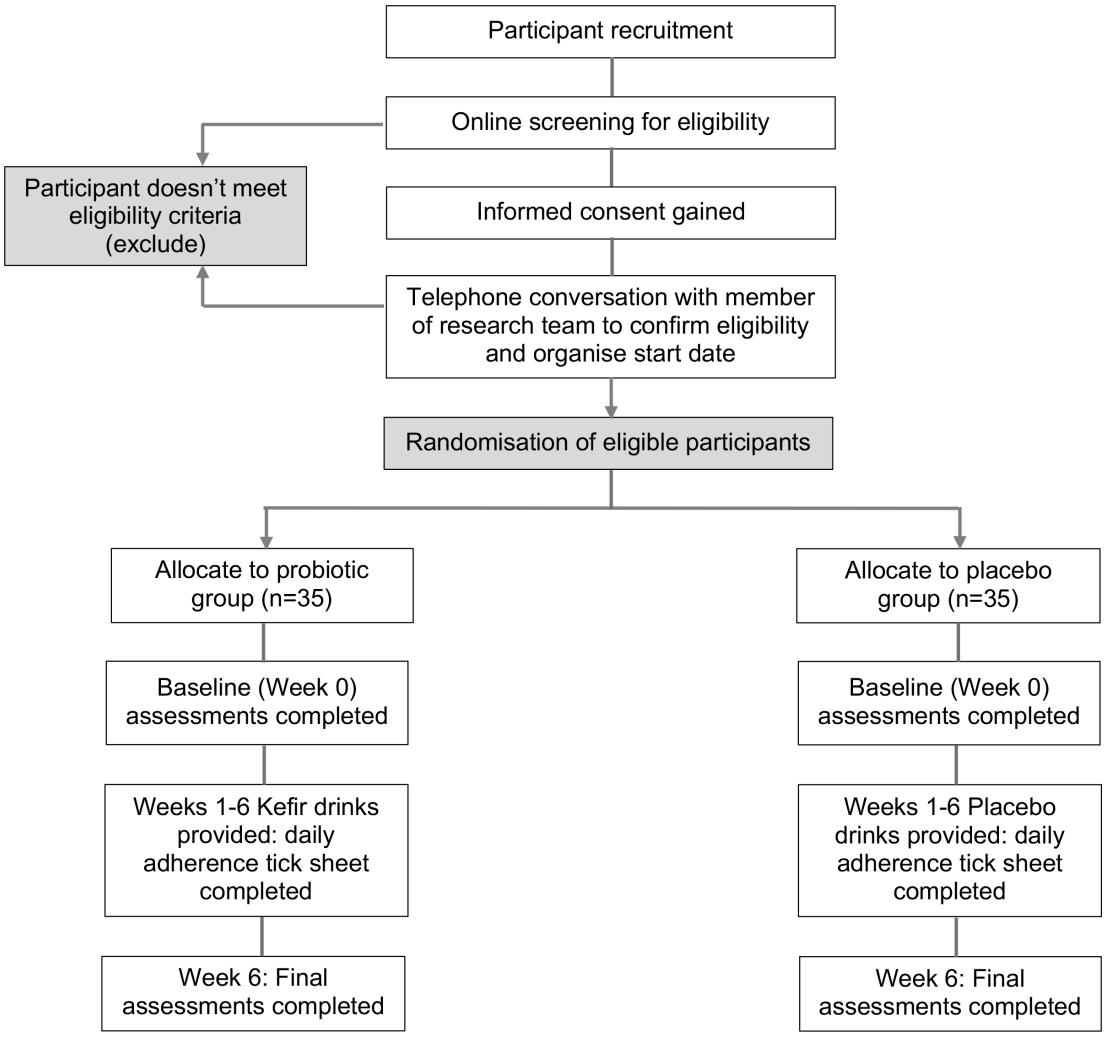

Figure 1 Flow diagram detailing ADHD study procedures. ADHD, attention-deficit/hyperactivity disorder.

Secondary outcome measures

Measure of impulsivity and inattention

The computerised Go/NoGo test will be used to measure impulsivity and inattention, core cognitive symptoms of ADHD.[57] Participants watch a sequential presentation of letters (presented in a 2×2 grid and respond to a target letter by pressing a key on the keyboard). The target letters P or R are randomly displayed in the grid for 500 ms. The interstimulus interval is 1500 ms, with 320 repetitions. In the first set of 160 trials, participants are required to respond when they see a P. In the second set of 160 trials, participants are requested to respond when they see an R. The ratio of targets to non-targets is 80:20 for both sets. The Go/NoGo test is hosted on the General Data Protection Regulation (GDPR) compliant website Gorilla.sc and completed online in participant's homes. Outcomes computed will be (1) Go errors (errors of omission), with a higher score indicating greater inattention; (2) NoGo errors (errors of commission), with a higher score indicating greater impulsivity; (3) Go reaction times, with a higher score indicating less impulsivity; (4) Go reaction time variability, with a higher score indicating greater inattention. This measure is sensitive to changes in ADHD symptoms in response to medication and exercise.[58 59]

Actigraphy recordings

Actigraphy provides an objective measure of sleep quality and daytime activity. Children will be provided with a 'Motionlogger Micro watch' (Ambulatory Monitoring) to wear on their non-dominant wrist for seven consecutive days.[60] Participants are instructed to remove the actigraphs only for swimming and contact sports. Off-wrist periods are marked as 'bad data' and not included in the analysis. Participants are requested to press an event-marker button on the watch when they attempt to get to sleep. The actigraphs record movement, temperature, light and physical activity during sleep and waking period.

Data from watches will be uploaded to a computer and analysed using Ambulatory Monitoring software, to obtain mean scores for the whole week. The actigraphy data are scored and analysed using the Sadeh algorithm using 'Action-W-V.2' software. Awakening rules will consider wake blocks as greater than or equal to 5 continuous minutes awake and end after 15 or more continuous minutes asleep. Movement data are recorded in 60 s

epochs, with zero-crossing mode movement sampling applied for sleep analysis. Sleep diary information, light and temperature data are used to detect and remove artefacts from the data and inform judgements in the event of a lack of clarity. The commencement of downtime is ascertained by finding a zero-crossing activity level greater than 200 and then moving one epoch towards the sleep period. Zero-crossing records the frequency of the signal crossing a particular threshold (set close to zero) during each epoch. The end of downtime is delimited by a waking zero-crossing activity level of greater than 200.

Outcomes computed will be: (1) Sleep duration (minutes); (2) Mean activity during sleep (higher score reflects more disrupted sleep). This value is derived using the total number of movement counts during the 'down-period' divided by the number of 60 s epochs; (3) Minutes spent awake during the down period (higher score reflects poorer quality sleep); (4) Sleep latency (minutes taken to fall asleep, higher score reflects more time taken to fall asleep); (5) Sleep efficiency (percentage down period spent asleep, excluding sleep latency, higher score reflects better quality sleep); (6) Wake after sleep onset (minutes spent awake during the down period after removing sleep latency (higher score reflects poorer quality sleep); (7) Sleep fragmentation (number of awakenings/total minutes of sleep×100), higher score reflects more fragmented sleep). Measures of mean and median daytime activity are computed using proportional integration mode sampling for wake-period activity.

Sleep diary

The Consensus Sleep Diary[61] is a short standardised tool for tracking nightly subjective sleep. Children are asked to complete this (with the assistance of their parents) for 7 days during week 0 of the study and again during the final week of the study. The diary takes approximately 3 min per day to complete and is used to detect and remove artefacts in the actigraphy data.

Sleep habits and disturbances

The Child's Sleep Habits Questionnaire (CSHQ)[62] and the Sleep Self Report (SSR)[62] assess participants' sleep habits. Both questionnaires use a 3-point scale to rate items relating to sleep retrospectively for the previous week. Parents are asked to complete the CSHQ, a 33-item questionnaire, with responses ranging from rarely (1) to usually (3), with some items reverse scored. A Total Sleep Disturbances score is calculated as the sum of all scored questions and can range from 33 to 99, with higher scores indicating more problematic sleep. Participants complete the SSR, a 26-item questionnaire including corresponding questions to the CSHQ. A Total Sleep Disturbances score is calculated as the sum of the 23 scored questions, and ranges from 23 to 69, with higher scores indicating more problematic sleep. The CSHQ and SSR have been assessed for validity in children diagnosed with ADHD,[62 63] with reasonable internal consistency.[64]

Gastrointestinal symptoms

The Gastrointestinal Severity Index (GSI) is used to assess changes in the gastrointestinal symptoms of participants during the intervention. The GSI is a validated tool for use in children aged 2–18 years and completed by the parent.[65] The index uses a 3-point rating scale (ranging from 0 to 2) across six gastrointestinal symptoms: constipation, diarrhoea, stool consistency, stool smell, flatulence, abdominal pain. Ratings are summed to provide a total score, ranging from 0 to 12 (with a high score indicating more severe symptoms). It has been found to be sensitive to detecting gastrointestinal symptoms in ADHD.[26]

Stool microbiome collection and analysis

Faecal samples will be collected in Shield faecal collection tubes (Zymo Research), delivered to participants at baseline and week six, together with gloves, cardboard bowl, ziplock bag and instructions. Parents are instructed to collect a sample of the child's faeces in the bowl, transfer a small amount to the tube, put the tube in the ziplock bag and return to the researchers in the prepaid packaging provided.

Samples will be stored locked in a lab at St Mary's University before being processed by SeqBiome (https://seqbiome.com/), to extract the faeces using the Qiagen QIAamp Fast DNA Stool Mini Kit (https://www.qiagen.com). Comparative changes in diversity and species (completion relative to baseline) are investigated using shotgun metagenomic microbiome sequencing. The resultant DNA is quantified using the Qubit double-stranded DNA high-sensitivity assay kit (Bio-Sciences, Dublin, Ireland). Samples are prepared for shotgun metagenomic sequencing according to Illumina DNA Prep library preparation kit guidelines, with the use of unique dual indexes for multiplexing with the Integrated DNA Technologies for Illumina index kit (https://eu.idtdna.com/). Final clean libraries are quantified by Qubit as before, and pooled using equimolar concentrations. Final sequencing pool quality check and quantification are performed by quantitative PCR using the KAPA Library Quantification Kit for Illumina (Roche KAPA). High-throughput sequencing is performed on a NextSeq 2000 platform using a P1 mid-output flow cell. The resultant data are quality checked and filtered using Kneaddata. The associated taxonomic profile is determined by Kraken2+Braken[66] and its functional potential determined using Humann3.[67]

Procedure

Participants will be assessed on all outcome measures at two time points. The first assessment (baseline) will occur prior to commencing the intervention. The second assessment will take place during the final week of intervention (week 6). A study pack (consisting of a stool sample collection kit, Motionlogger Micro Watch actigraph, sleep diary, daily adherence chart, recipe suggestions and full instructions) will be delivered to the participants home in advance of assessment weeks and families will be

provided with a prepaid signed for Royal Mail envelope in which to return the actigraph, stool sample and daily adherence chart at the end of the week. Parents are asked to complete a short background questionnaire about variables such as ethnicity, type of delivery at birth, antibiotic usage, etc. In order to promote retention, families will be contacted throughout the trial to check on progress, answer any questions, offer support, prompt completion of any outcome measures and encourage compliance. Any modifications to the study will require approval from the funding body and the Ethics Committee, prior to implementation. The ClinicalTrials.gov record will also be amended accordingly. Data collection for all questionnaires uses the JISC Online surveys platform (https://www.onlinesurveys.ac.uk) to ensure data are stored in a secure and GDPR compliant environment.

Statistical analysis

All randomised participants with valid baseline and endpoint data will be included in the analysis. The plan does not use intention-to-treat analysis, as we are assuming that if participants drop out, it will be highly unlikely that we are able to collect endpoint data from them. All outcome variables will be checked for normality and outliers. If the distribution is not normal, a suitable transformation will be used to create normally distributed variables. All subsequent analyses will be conducted on normally distributed variables. Interaction between time (preintervention/postintervention) and group (probiotic/placebo) is analysed using a Mixed Model ANOVA. The subject is the random effect in the model, the treatment is the fixed effect. Age group, gender, medication status and pretreatment value of the outcome variable will be used as covariates. All main effects and interactions will be assessed in the first model. Non-significant interactions will be removed, starting with highest order interactions and the resulting model will be compared with the previous model using Akaike information criterion (AIC),[68] where a smaller AIC value indicates a better model. Models will be chosen on the basis of 'best fit' and interaction terms that improve the fit will be retained.[69] First order interactions between treatment and covariates will be added to the model. Non-significant interactions and covariate effects will be removed from the final model. Post hoc t-tests will be used to further explore any significant results.

Data management and monitoring

Participant confidentiality will be ensured by allocating a participant identification code to each participant as a unique identifier for all data collection. The participants' name, address, phone number and email address along with the unique numerical identifier will be stored separately and collected only for the purpose of distributing the actigraphs, microbiome kits, surveys and intervention drink as well as tracking consent and managing withdrawal requests. The spreadsheet containing the unique identifier linked to the participant details will be stored in a password-protected file on the St Mary's university servers and only accessible by approved research team members. All data collected uses the unique identifier and does not contain personal identifying information. The data will be monitored by the research team without the need for a further data monitoring committee. There will be no interim analysis of data and termination of the trial would only occur due to unanticipated adverse events. The principal investigator will audit data at least once per month, for overall completeness, quality and recording of adverse events.

Once the collection period has been completed, the data will be held on St Mary's University servers securely for a period of at least 10 years. Access to the data will be limited to the research team as required. Once anonymised, data will be made available to researchers via accessible data repositories and possibly used for novel purposes. Any publications resulting from this study will be done with all participant data anonymised so that it will not be possible to identify participants from the report. After data analysis, participants will be sent a report outlining the key findings, together with being informed of their group allocation (probiotic/placebo).

Ethics and dissemination

Ethical approval for the study was granted by St Mary's University Ethics Committee (SMU_ETHICS_2020-21_240) and parents and children will be provided with full written information about the study before written consent and assent are obtained (online supplemental file 1). Kefir is generally well tolerated, although potential adverse effects of consumption include gastrointestinal symptoms, such as bloating and flatulence.[51] To reduce any risk to vulnerable individuals, participants with a milk allergy or lactose intolerance; those who have a diagnosis of a gastrointestinal disorder, for example, inflammatory bowel disease or coeliac disease; and those who have a diagnosis of an autoimmune disease or compromised immunity, will be excluded from taking part. In addition, we will operate an adverse event recording and management plan (detailed previously), with side effects and adverse events being recorded and monitored throughout the study. Findings will be disseminated through journal articles and presentations at both academic/medical conferences and to community groups. Authorship of articles will be determined by discussion within the research team, adhering to authorship guidelines.

DISCUSSION

There is much interesting research concerning the use of dietary interventions in ADHD, but the most effective diets are highly restrictive, with limited understanding of the mechanisms underpinning their success.[22] We propose that dietary interventions may work, in part, because of the positive impact they have on the gut microbiome. Burgeoning evidence exists to suggest that kefir can have

a positive impact on microbiome composition.[29–32] By employing a double-blind, placebo-controlled design, this study will be the first to evaluate the effects of kefir on behaviour, sleep, gastrointestinal symptoms and the microbiome in children with ADHD.

With many dietary interventions, it is not possible to easily blind the participants, or researchers to whether they have been allocated to the intervention or placebo group. The consumption of kefir as a supplement to diet, overcomes some of these difficulties as neither participants or researchers will be aware of group allocation, which is critical to minimise expectation bias. Conducting the study could have a practical impact, as the intervention would be very easy to implement in the real-world. Kefir is readily available from many supermarkets and online suppliers and its consumption does not require direction from a specialist practitioner. It could be made more cost-effective by individuals making their own kefir, which is simple, low-cost and does not require any specialist equipment or skills.[70]

To conclude, this study will advance our understanding of any potential impact of kefir on ADHD symptoms in children. It will also allow exploration of a potential mechanism by which kefir may exert an effect on behaviour by exploring microbiota changes as a result of the intervention. This study has the potential to provide evidence for the use of kefir as a cost-effective, easily implemented dietary intervention, adjunct to standard care, for children diagnosed with ADHD.

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Contributors Conceptualisation: KL, AMG, MAT-M, KM, PF, JH, FQ and PDC; writing—original draft preparation: KL and PF; writing—review and editing: KL, AMG, MAT-M, KM, PF, JH, FQ and PDC; project administration: KL, PF and JH; funding acquisition: KL and AMG. All authors have read and agreed to the published version of the manuscript.

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Competing interests Nourish Kefir will supply organic cow's milk kefir for the study at a reduced cost. They exert no control over the publication of results. AMG is an advisor for a project initially sponsored by Johnson's Baby. She is a consultant for Perrigo (2021+). She receives royalties for two books Nodding Off (Bloomsbury Sigma, 2018) and The Sleepy Pebble (Flying Eye, 2019). She has another contract with Lawrence King Publishers (publication due 2023). She was previously a CEO of Sleep Universal LTD (2022). She is a regular contributor to BBC Focus Magazine and has contributed to other outlets (such as The Conversation, The Guardian and Balance Magazine). She occasionally receives sample products related to sleep (eg, blue light blocking glasses) and has given a paid talk to a business (Investec). She is a specialist subject editor at JCPP (sleep) for which she receives a small honorarium. She has contributed a paid article to Neurodiem. KL previously held a paid role as Research Editor for Foodsmatter. She is an Editorial Board Member for the British Association of Nutritional and Lifestyle Medicine, Nutritional Evidence Database (NED) and a Scientific Advisory Board Member for Chuckling Goat, both in an unpaid capacity. She is occasionally paid, or receives hospitality, to deliver talks on her research and infrequently receives sample products related to health and nutrition. PDC is a cofounder and is the CTO of SeqBiome. He has also been occasionally paid, or received hospitality, to deliver talks on his research. MAT-M is Head of R&D for Chuckling Goat, and was previously employed by Atlas Biomed as Director of Health and Nutrition Research.

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REFERENCES

1 Sayal K, Prasad V, Daley D, *et al*. ADHD in children and young people: prevalence, care pathways, and service provision. *Lancet Psychiatry* 2018;5:175–86.
2 American Psychiatric Association. *Diagnostic and statistical manual of mental disorders*5th ed. 22 May 2013.
3 Punja S, Shamseer L, Hartling L, *et al*. Amphetamines for attention deficit hyperactivity disorder (ADHD) in children and adolescents. *Cochrane Database Syst Rev* 2016;2.
4 Klein RG, Mannuzza S, Olazagasti MAR, *et al*. Clinical and functional outcome of childhood attention-deficit/ hyperactivity disorder 33 years later. *Arch Gen Psychiatry* 2012;69:1295.
5 Owens JA. Sleep disorders and attention-deficit/hyperactivity disorder. *Curr Psychiatry Rep* 2008;10:439–44.
6 Mohammadi MR, Mostafavi SA, Keshavarz SA, *et al*. Melatonin effects on children with Adhd melatonin effects in methylphenidate treated children with attention deficit hyperactivity disorder: a randomized double blind clinical trial. 2012. Available: www.tums.ac.ir
7 Bendz LM, Scates AC. Melatonin treatment for insomnia in pediatric patients with attention-deficit/hyperactivity disorder. *Ann Pharmacother* 2010;44:185–91.
8 Yürümez E, Kılıç BG. Relationship between sleep problems and quality of life in children with ADHD. *J Atten Disord* 2016;20:34–40.
9 Becker SP. ADHD and sleep: recent advances and future directions. *Curr Opin Psychol* 2020;34:50–6.
10 Peasgood T, Bhardwaj A, Biggs K, *et al*. The impact of ADHD on the health and well-being of ADHD children and their siblings. *Eur Child Adolesc Psychiatry* 2016;25:1217–31.
11 Doshi JA, Hodgkins P, Kahle J, *et al*. Economic impact of childhood and adult attention-deficit/hyperactivity disorder in the United States. *J Am Acad Child Adolesc Psychiatry* 2012;51:990–1002.
12 Le HH, Hodgkins P, Postma MJ, *et al*. Economic impact of childhood/adolescent ADHD in a European setting: the Netherlands as a reference case. *Eur Child Adolesc Psychiatry* 2014;23:587–98.
13 Storebø OJ, Ramstad E, Krogh HB, *et al*. Methylphenidate for children and adolescents with attention deficit hyperactivity disorder (ADHD). *Cochrane Database Syst Rev* 2015.

14 National Institute for Health and Care Excellence (NICE). Attention deficit hyperactivity disorder: diagnosis and management. 2022. Available: https://www.nice.org.uk/guidance/ng87
15 Arnold LE, Lofthouse N, Hurt E. Artificial food colors and attention-deficit/hyperactivity symptoms: conclusions to dye for. *Neurotherapeutics* 2012;9:599–609.
16 Sonuga-Barke EJS, Brandeis D, Cortese S, *et al*. Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. *Am J Psychiatry* 2013;170:275–89.
17 Hurt EA, Arnold LE, Lofthouse N. Dietary and nutritional treatments for attention-deficit/hyperactivity disorder: current research support and recommendations for practitioners. *Curr Psychiatry Rep* 2011;13:323–32.
18 Puri BK, Martins JG. Which polyunsaturated fatty acids are active in children with attention-deficit hyperactivity disorder receiving PUFA supplementation? A fatty acid validated meta-regression analysis of randomized controlled trials. *Prostag Leukot Ess Fatty Acids* 2014;90:179–89.
19 Sonuga-Barke EJS, Brandeis D, Cortese S, *et al*. Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of. *Am J Psychiatry* 2013;170:275–89. 10.1176/appi.ajp.2012.12070991 Available: http://www.crd.york.ac.uk/prospero/
20 Pelsser LM, Frankena K, Toorman J, *et al*. Diet and ADHD, reviewing the evidence: a systematic review of meta-analyses of double-blind placebo-controlled trials evaluating the efficacy of diet interventions on the behavior of children with ADHD. *PLoS One* 2017;12.
21 Benton D. The impact of diet on anti-social, violent and criminal behaviour. *Neurosci Biobehav Rev* 2007;31:752–74.
22 Lawrence K, Myrissa K, Toribio-Mateas M, *et al*. Trialling a Microbiome-targeted dietary intervention in children with ADHD-the rationale and a non-randomised feasibility study. *Pilot Feasibility Stud* 2022;8:108:108.:.
23 Prehn-Kristensen A, Zimmermann A, Tittmann L, *et al*. Reduced microbiome alpha diversity in young patients with ADHD. *PLoS One* 2018;13.
24 Aarts E, Ederveen THA, Naaijen J, *et al*. Gut Microbiome in ADHD and its relation to neural reward anticipation. *PLoS One* 2017;12:e0183509.
25 Bull-Larsen S, Mohajeri MH. The potential influence of the bacterial Microbiome on the development and progression of ADHD. *Nutrients* 2019;11:2805.
26 Ming X, Chen N, Ray C, *et al*. A gut feeling: a hypothesis of the role of the Microbiome in attention-deficit/hyperactivity disorders. *Child Neurol Open* 2018;5.
27 Bourrie BCT, Willing BP, Cotter PD. The Microbiota and health promoting characteristics of the fermented beverage Kefir. *Front Microbiol* 2016;7:647.
28 Simova E, Beshkova D, Angelov A, *et al*. Lactic acid bacteria and yeasts in Kefir grains and Kefir made from them. *J Ind Microbiol Biotechnol* 2002;28:1–6.
29 Liu J, Wang S, Chen M, *et al*. The anti-allergenic properties of milk Kefir and soymilk Kefir and their beneficial effects on the intestinal microflora. *J Sci Food Agric* 2006;86:2527–33. 10.1002/jsfa.2649 Available: https://onlinelibrary.wiley.com/toc/10970010/86/15
30 van de Wouw M, Walsh AM, Crispie F, *et al*. Distinct actions of the fermented beverage Kefir on host behaviour, immunity and microbiome gut-brain modules in the mouse. *Microbiome* 2020;8:67.
31 Bellikci-Koyu E, Sarer-Yurekli BP, Akyon Y, *et al*. Effects of regular kefir consumption on gut microbiota in patients with metabolic syndrome: a parallel-group, randomized, controlled study. *Nutrients* 2019;11:2089.
32 Yılmaz İ, Dolar ME, Özpınar H. Effect of administering kefir on the changes in fecal microbiota and symptoms of inflammatory bowel disease: a randomized controlled trial. *Turk J Gastroenterol* 2019;30:242–53. 10.5152/tjg.2018.18227 Available: https://www.turkjgastroenterol.org/EN/march-2019-00313
33 Hsu Y-J, Huang W-C, Lin J-S, *et al*. Kefir supplementation modifies gut microbiota composition, reduces physical fatigue, and improves exercise performance in mice. *Nutrients* 2018;10:862.
34 ChifiriucMC, Cioaca AB, Lazar V. In vitro assay of the antimicrobial activity of kephir against bacterial and fungal strains. *Anaerobe* 2011;17:433–5.
35 Silva KR, Rodrigues SA, Filho LX, *et al*. Antimicrobial activity of broth fermented with Kefir grains. *Appl Biochem Biotechnol* 2009;152:316–25.
36 Feingold BF. *Attention-deficit hyperactivity disorder, Food additives, Hyperkinesis*. New York: Random House, 1975.
37 Howard AL, Robinson M, Smith GJ, *et al*. ADHD is associated with a 'Western' dietary pattern in adolescents. *J Atten Disord* 2011;15:403–11.
38 Nigg JT, Lewis K, Edinger T, *et al*. Meta-analysis of attention-deficit/hyperactivity disorder or attention-deficit/hyperactivity disorder symptoms, restriction diet, and synthetic food color additives. *J Am Acad Child Adolesc Psychiatry* 2012;51:86–97.
39 Pellow J, Solomon EM, Barnard CN. Complementary and alternative medical therapies for children with attention-deficit/hyperactivity disorder (ADHD). *Altern Med Rev* 2011;16:323–37.
40 Wilson RF, Tyburski JG. Metabolic responses and nutritional therapy in patients with severe head injuries. *J Head Trauma Rehabil* 1998;13:11–27.
41 Kaplan BJ. The emerging field of nutritional mental health: inflammatio, the microbiome, oxidative stress. In: *Inflammation, the Microbiome, Oxidative Stress, and Mitochondrial Function*. 2020.
42 Chan A-W, Tetzlaff JM, Altman DG, *et al*. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.
43 Suresh K. An overview of randomization techniques: an unbiased assessment of outcome in clinical research. *J Hum Reprod Sci* 2011;4:8–11.
44 Hu F, Hu Y, Ma Z, *et al*. Adaptive randomization for balancing over covariates. *WIREs Computational Stats* 2014;6:288–303. 10.1002/wics.1309 Available: https://wires.onlinelibrary.wiley.com/toc/19390068/6/4
45 Smoak CG, Lin JS. A SAS program to perform adaptive randomization. Statistics, Data Analysis, and Data Mining; 1998:242–6 Available: https://support.sas.com/resources/papers/proceedings/proceedings/sugi26/p242-26.pdf
46 Reale L, Bartoli B, Cartabia M, *et al*. Comorbidity prevalence and treatment outcome in children and adolescents with ADHD. *Eur Child Adolesc Psychiatry* 2017;26:1443–57.
47 ClementHW, Pejovic-Milovancevic M, Schmeck K. A pilot randomized control trial with the probiotic strain lactobacillus rhamnosus GG (LGG). 2020;1:181.
48 Polderman TJC, Derks EM, Hudziak JJ, *et al*. Across the continuum of attention skills: a twin study of the SWAN ADHD rating scale. *J Child Psychol Psychiatry* 2007;48:1080–7.
49 Arnett AB, Pennington BF, Friend A, *et al*. The SWAN captures variance at the negative and positive ends of the ADHD symptom dimension. *J Atten Disord* 2013;17:152–62.
50 Stevenson J, Buitelaar J, Cortese S, *et al*. Research review: the role of diet in the treatment of attention-deficit/hyperactivity disorder--an appraisal of the evidence on efficacy and recommendations on the design of future studies. *J Child Psychol Psychiatry* 2014;55:416–27.
51 Williams NT. Probiotics. *Am J Health Syst Pharm* 2010;67:449–58.
52 U.S. Department of Health and Human Services NI, Institute of H and NC. Common terminology criteria for adverse events (CTCAE) version 5.0. 2017. Available: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_8.5x11.pdf
53 European Commission. Communication from the Commission – detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use ('CT-3. *Official Journal of the European Union* 2011. Available: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2011:172:0001:0013:EN:PDF
54 Swanson JM, Schuck S, Porter MM, *et al*. Categorical and dimensional definitions and evaluations of symptoms of ADHD: history of the SNAP and the SWAN rating scales. *Int J Educ Psychol Assess* 2012;10:51–70.
55 BurtonCL, WrightL, ShanJ. Utility of the SWAN scale for ADHD trait-based genetic Researchfile:///C:/Users/04585/Downloads/Burton_2019_Swan_Validity.Pdf: A validity and Polygenic risk study; 2018. bioRxiv.
56 Burton CL, Wright L, Shan J, *et al*. SWAN scale for ADHD trait-based genetic research: a validity and polygenic risk study. *J Child Psychol Psychiatry* 2019;60:988–97.
57 Bezdjian S, Baker LA, Lozano DI, *et al*. Assessing inattention and Impulsivity in children during the go/nogo task. *Br J Dev Psychol* 2009;27(Pt 2):365–83.
58 Chuang LY, Tsai YJ, Chang YK, *et al*. Effects of acute aerobic exercise on response preparation in a go/no go task in children with ADHD: an ERP study. *J Sport Health Sci* 2015;4:82–8.
59 Horowitz I, Avirame K, Naim-Feil J, *et al*. The interactive effects of test-retest and methylphenidate administration on cognitive performance in youth with ADHD: a double-blind placebo-controlled crossover study. *Psychiatry Res* 2020;291:113056.

60 Denis D, Eley TC, Rijsdijk F, *et al*. Is Digital cognitive behavioural therapy for insomnia effective in treating sub-threshold insomnia: a pilot RCT. *Sleep Med* 2020;66:174–83.
61 Carney CE, Buysse DJ, Ancoli-Israel S, *et al*. The consensus sleep diary: standardizing prospective sleep self-monitoring. *Sleep* 2012;35:287–302.
62 Owens JA, Maxim R, Nobile C, *et al*. Parental and self-report of sleep in children with attention-deficit/hyperactivity disorder. *Arch Pediatr Adolesc Med* 2000;154:549–55.
63 Owens JA, Spirito A, McGuinn M. The children's sleep habits questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. *Sleep* 2000;23:1043–51.
64 Gregory AM, Van der Ende J, Willis TA, *et al*. Parent-reported sleep problems during development and self-reported anxiety/depression, attention problems, and aggressive behavior later in life. *Arch Pediatr Adolesc Med* 2008;162:330–5.
65 Thulasi V, Steer RA, Monteiro IM, *et al*. Overall severities of gastrointestinal symptoms in pediatric outpatients with and without autism spectrum disorder. *Autism* 2019;23:524–30.
66 Lu J, Rincon N, Wood DE, *et al*. Metagenome analysis using the kraken software suite. *Nat Protoc* 2022;17:2815–39.
67 Beghini F, McIver LJ, Blanco-Míguez A, *et al*. Integrating Taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. *Elife* 2021;10:e65088.
68 Akaike H. A new look at the statistical model identification. *IEEE Trans Automat Contr* 1974;19:716–23.
69 Portet S. A primer on model selection using the akaike information criterion. *Infect Dis Model* 2020;5:111–28.
70 Alves E, Ntungwe EN, Gregório J, *et al*. Characterization of Kefir produced in household conditions: Physicochemical and nutritional profile, and storage stability. *Foods* 2021;10:1057.