# Physiological and self-reported effects of Kombucha on well-being, induced stress, and inflammation (INBK)

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## 1) Introduction

Epidemiological studies have linked tea consumption to lower levels of emotional distress. Probiotic, fermented products such as kombucha, (fermented with a symbiotic culture (scoby) of bacteria and yeasts), have attracted significant interest due to their potential beneficial health-giving and wellness properties. Kombucha has multiple functional properties, including promoting favourable intestinal microbiome colonisation to improve gastrointestinal function, and anti-inflammatory, antioxidant, and metabolic activity including reduced cholesterol levels and blood pressure. This project will randomise individuals into the intervention (kombucha) or placebo (flavoured water) arm for 8 weeks and seeks to assess physiological and self-reported effects of Kombucha on well-being, induced stress, and inflammation.

## 2) Statement of Purpose

A randomised, double-blinded, placebo-controlled, 2-arm parallel human clinical trial assessing kombucha consumption for 8-weeks on well-being, working memory, and stress, using self-report psychometric and behavioural tasks in addition to physiological assessments of salivary cortisol, metabolomics, and inflammation.

## 3) Investigational Product

### 3.1) Description

A cohort (n = 60) of adults (18-60) who will be randomised into Kombucha or placebo supplementation (330ml a day) for 8 weeks. Subjects will be asked to take the kombucha across the course of the day. The placebo will be a carbonated drink matched to the active product by taste and texture.

**Kombucha**
Water, Live Kombucha Culture*, Cane Sugar*, Green Tea*, Black Tea*. *organic

**Placebo**
Water, Cherry Syrup 2% [Sour Cherry Juice Concentrate (2.29%), Red Grape Juice (2.23%), Preservative: (Malic Acid), Natural Colour: (Carantho), Natural Flavouring: (Red Grape, Sour Cherry), Preservative: (Potassium Sorbate), Antioxidant (Ascorbic Acid (Vitamin C)), Hibiscus]

### 3.2) Quality

The kombucha and placebo were prepared using food-grade ingredients and prepared and packed in a food-grade unit.

### 3.3) Dose

330ml a day of Kombucha or placebo (carbonated drink matched to the active product by taste and texture) in a can to be consumed across the course of the day, for 8-weeks.

## 4) Investigational Product Safety

The Kombucha contains green tea, which contains low amounts of caffeine. The products are not hazardous. The products are free of the following components and their products thereof: cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk (including lactose), nuts, celery, mustard, sesame seeds, sulphur dioxide and sulphite, lupin and molluscs, in compliance with Regulation (EC) No. 1169/2011. The products do not contain added artificial colorants or E numbers. The kombucha and placebo are vegan, and they are produced and stored in a food-grade facility.

## 5) Study Design

### 5.1) Objectives of the Study

The **primary objective** of this proposal is to test our hypothesis that daily Kombucha consumption over 8 weeks in a healthy human cohort will improve quality of life as measured by the EuroQol - 5D-5L from baseline and when compared with placebo.

A **secondary objective** of this proposal is to test our hypothesis that daily Kombucha consumption over 8 weeks in a healthy human cohort, will reduce stress (as measured by cortisol concentrations in saliva) after participation in the MAST, when compared to baseline measurements and the placebo control group.

Other **secondary objectives** include:

Psychological Well-Being is measured by the Carol Ryffs (1989) Psychological Well-Being (PWB) Scale (18-item) at baseline and after 8 weeks, in both study arms.

Emotional states of depression, anxiety and stress are measured by the Depression Anxiety Stress Scale (DASS; 21-item) at baseline and after 8 weeks, in both study arms.

Mood or emotion are measured by the Positive and Negative Affect Scale (PANAS)-GEN at baseline and after 8 weeks, in both study arms.

Pain will be measured by the Visual Analogue Scale (VAS) at baseline and after 8 weeks, in both study arms.

Near global metabolomics of first-morning void urine will be measured by flow injection using electro-spray mass spectrometry instrumentation at baseline, and after 8 weeks, in both study arms.

Near global metabolomics of fasting plasma will be measured by flow injection using electro-spray mass spectrometry instrumentation at baseline, and after 8 weeks, in both study arms.

Inflammation will be measured by cytokine analysis (TFN-alpha) of fasting serum using Elizas at baseline, and after 8 weeks, in both study arms.

### 5.2) Subject Selection

60 participants from Ceredigion and surrounding areas, between 18 and 60 years of age, mixed gender, mixed ethnicity.

### 5.3) Inclusion Criteria:

- Subjects over 18 years of age and under 60 years of age
- Subjects who are able to commit to multiple visits to WARU (Aberystwyth University)
- Subjects who can provide venous blood samples, urine samples, and saliva samples.
- Subjects able to provide written informed consent prior to performing any study procedures.

### 5.4) Exclusion Criteria

- Subjects with a diagnosis of Alzheimer's disease or other forms of dementia
- Subjects taking medication for the treatment of dementia (such as acetylcholinesterase inhibitors (Aricept, Excelon), memantine (Namenda) or other medications with similar mechanisms of action) or medical foods (such as Cerefolin, Souvenaid, Axona) for the treatment of dementia.
- Subjects who are pregnant or lactating
- Subjects with medical condition or disease that is life-threatening
- Subjects diagnosed with diabetes.
- Subjects already consuming pro-biotics who will not comply with the 4-week washout
- Subjects with any cardiovascular diseases
- Subjects with severe physical illnesses (e.g., fibromyalgia)
- Subjects experiencing hypertension (high blood pressure)
- Subjects with endocrine disorders
- Subjects suffering from substance abuse
- Subjects who heavily smoke (>10 cigarettes/day)
- Any medication known to affect the HPA axis

### 5.5) Study Design

**Induction**

If the participant passes the eligibility checks (done in-person or over the phone), we will invite the participant to attend an in-person induction session at WARU. During this induction, if happy to

proceed, we will ask the participant to sign a consent form and to complete a medical health questionnaire. The participant will also be instructed on how to use our at-home urine sampling kits, which will be handed to the participant during this appointment. We will also request to measure the participant's height, weight, waist circumference, and hip circumference. The participant will be randomised into the intervention or placebo arm of the trial (double blinded however the participant can ask to find out at the end of the trial).

**Visit 1 (split or combined)**

On the participant's next visit to WARU, they will arrive fasted for their venous blood draw (followed by optional tea and toast). They will also bring their first Morning Void urine samples with them. On the same day (in the afternoon), or within 48 hours (this will be arranged with the researcher prior to the participant's arrival), we will measure the participants heart rate and blood pressure and ask that the participant complete self-report questionnaires (the Carol Ryffs (1989) Psychological Well-Being (PWB) Scale (18-item), the Depression Anxiety Stress Scale (DASS; 21-item), the Positive and Negative Affect Scale (PANAS)-GEN, the Visual Analogue Scale (VAS), and the EQ5D-5L, and the PANAS-NOW).

*Please note that if the participant typically experiences high blood pressure between 135/85 mmHg to 150/95 mmHg at home, or if we observe a reading of 140/90 mmHg and 160/100 mmHg in the lab (on test day 1), then the participant will unfortunately not be eligible to participate. These numbers have been taken from the British Heart Foundation (March 2023). This is because we anticipate participants to experience short-term elevations in blood pressure during the hand-immersion trials of the MAST.*

The participant will have to wait an hour between when the participant last consumed food or water, to when the participant's first saliva sample is taken (required as a measure of objective well-being state). Once the first saliva sample has been taken (which the researchers will guide the participant through), the participant will be asked to roll up their sleeves, remove any jewellery, and to wash their hands and arms with soap and water in preparation for the Maastricht Acute Stress Test (MAST).

The MAST involves the participant completing a series of ice water (2$^{o}$C) submersion of the participants hand (up to the participants wrist) in a controlled manner for short periods over a 15-minute duration. Submersion is expected to cause manageable levels of acute pain, all of which will be within the participants control because the participant can remove the participants hand at any point (although for the purpose of the MAST, the participant will be encouraged to wait for the signal on the screen). During the period of the task, the participant will see instructions on a screen of when to immerse or removed the participants hand and also be asked to complete some basic arithmetic tasks. During this task the participant will also be connected to passive recording devices to measure the participants heart rate and electrical levels from the participants skin (known as electrodermal activity). The participant will also be fake-video recorded during the experiment as part of the protocol. Immediately after the MAST is completed the participant will complete some more questionnaires (PANAS-NOW and visual analogue scale (VAS) to report how stressful, painful, and unpleasant the MAST was (scale = 0 = ‘‘not at all’’ to 100 = ‘‘extremely’’)) and provide saliva samples at set times (T0m, T10m, T20m, T30m, T40m).

After the testing and samples have been collected the participant will be provided with the first week or two of trial drinks and the participant will be asked to consume 330ml throughout the course of the day (a whole bottle), every day, for 8 weeks. During this time, the researchers will be in touch (via phone or email, whichever the participant prefers) to see how they are doing. Additionally, the researchers will arrange a weekly or biweekly appointment for the participant to collect the kombucha/placebo samples.

After the 8-week intervention, the participant will be invited to return for the final testing day with their urine samples. The participant will be asked to repeat the same series of events as those completed during testing day 1, and a separate day (within 48 hours of day 56) will be arranged for the venous blood draws.

There will be an optional feedback questionnaire at the end, and the participant will be informed about which group they were in (kombucha or carbonated placebo).

## 6) Participant Risks

The MAST is designed to create low levels of acute, manageable pain via temperatures of 2ºC via a cold pressor i.e., temperature-controlled ice water. To ensure that the pain levels are suitable for this type of experiment and to ensure the safety of participants, the same procedure developed by Smeets et al., 2012 will be used. In addition, participants will remain completely in control of their hand submersion, so if the pain becomes too much, they can easily withdraw their hand from the water. If they decide they would like to continue with the MAST, they can choose to submerge their hand again in the water during the next submersion interval, and continue doing so (submerging and retrieving) until the end of the experiment. The researcher will make a note of any 'failed' submersion trials. Participant heart rate will be continuously monitored throughout the MAST, so if participant HR exceeds the upper threshold, then the experiment will be stopped. The upper threshold limit for beats per minute will be calculated for each participant using the following equation: (Beats per minute – Age of participant)/60 seconds = upper threshold limit for beats per minute. Participants will be explained the procedure prior to taking part and be allowed to ask any questions before taking part to provide informed consent. They will also be reminded of their right to withdraw and to stop the experiment at any point. Additionally, participants shall be given thorough instructions before they are requested to provide urine, blood, and saliva samples. If participants wish to no longer participate in the MAST, but still wish to continue with the rest for the trial (providing bloods, urines and questionnaire data and consuming the kombucha/placebo for the 8-week intervention period), then they will be permitted to do so.

Due to the nature of the topics assessed in this study participants will be given contact details for Mind, the Samaritans, MentalHealth.org and the student well-being services in the participant information sheet (PIS) and debrief sheet. There will also be first aid-trained individuals available throughout the study to account for any pain-related issues that may arise due to exceptional circumstances beyond the known level of pain induction from the cold pressor.

Deception: Part of the Mast protocol is to state to participants that they are being video recorded, but they will not be. It is done to increase levels of stress, based on findings that the perception of being videotaped whilst performing a mental arithmetic task reliably elicits strong neuroendocrine stress responses, including a 2- to 3-fold increase in salivary cortisol concentrations, rendering it an essential (and therefore necessary) social-stress component of the MAST protocol to stimulate the HPA axis (Dickerson and Kemeny, 2004, as cited by Smeets et al., 2012). This choice of, and reason for, deception will be explained to participants in person, during their debrief, at the end of session 2. Participants will have plenty of time to ask questions to ensure they are happy with how all components of the study were conducted.

## 7) Benefits to participant

Participants who choose to take part and complete all sessions will be awarded £20 for every session they spend on campus. If they are an Aberystwyth University Psychology student, then they will also be awarded 5 SONA credits for each completed visit.

In addition to the financial incentive, by participating in this research, they will allow researchers to gain important insight into the effects of kombucha on well-being, working memory, and inflammation, which will strengthen our understanding of the physiological benefits of post-biotics on the gut and gut-brain axis.

**8) Privacy/confidentiality**

Participants are informed that only the researchers involved with the study will be able to look at the information they provide. Specific details and personal identifiers will only be available to the researchers. REDCap will be used, which is a secure web application for building and managing online surveys and databases which can be used to collect any data in compliance with GDPR. At the end of the study, any information relating to participants will be made pseudonymous (coded without their name associated). Participants will not be identifiable in any publication that may arise from this research. Electronic files will be kept in a logical manner and will always be kept grouped within specific folders and password protected. All data storage is using the AU network and backed up. Once the raw data has been extracted from a paper version onto a computer, the paper will be destroyed via a paper shredder or in confidential waste bags to ensure the participant's confidentiality. There may be times when keeping paper forms are necessary (consent forms), but in this case the paper versions will be kept in a locked filing cabinet. All files that are stored on the WARU share drive will always be protected with a secure password. Setting passwords will automatically encrypt the document and will not allow any unauthorised access to the data. The Gatekeeper of the passwords delivers the passwords by encrypted emails. When sharing data, the researchers will be using the WARU shared drive, which will be kept organised, and all documents will be in relevant folders and password protected if containing confidential information. The only people that will have access to the share drive are WARU staff, directly involved with the project. When sharing data between researchers, this will be done by uploading the document to the WARU share drive. Documents will not be sent directly via email. All biofluids are stored in a locked freezer. Key is kept by the gatekeeper.

**9) Safety Monitoring**

**9.1) Participant:**

If a participant, or a member of their family/household become unwell during the study, then they are pre-warned to alert a member of the research team immediately using the contact information they have been provided. Participation in the study will be suspended immediately until further discussion with the research team has taken place. If they become unwell at any point and need medical assistance, they are advised to contact 111 and seek advice from the NHS health sector or their doctor's surgery. The researchers have a duty of care towards them and can help monitor their health remotely over 14 days and will help in any way they can.

**9.2) Data:**

REDCap will be used, which is a secure web application for building and managing online surveys and databases which can be used to collect any data in compliance with GDPR. Each computer that belongs to the WARU team is programmed with the university network. Therefore, to gain access to the computer each member of staff will have a unique password to gain access to their own desktop account. Staff members are always expected to lock their computers when not attended. The WARU email account is only accessed by authorised individuals. The Gatekeeper regularly changes the password to enable access. Email addresses of study participants will be recorded, and study specific emails will be diverted to a secure named folder, only accessed by the immediate WARU Team. Each Aberystwyth University computer is scheduled to perform daily updates for anti-virus and firewall protection, this aims at keeping the university network, bug, and virus free. In the event of a power surge the university's network is backed up every 15 minutes therefore content is regularly saved automatically on the universities back up drive. Confidential data will not be stored on mobile devices (phones, laptops, tablets). If for any reason confidential data needs to be stored on a mobile device then permission must be gained from the line manager first, and if granted, all documents will be encrypted. For participant confidentiality, all personal data such as names, addresses, DOB and phone numbers, will be stored and password protected and there will be a gatekeeper of the password. Personal details will be removed or

replaced with an ID code when data is being analysed. Only the gatekeeper can link names to codes. Sensitive materials (participant's details e.g., address) will be electronically stored away and access will be restricted. This data will be password protected and there will be a gatekeeper of the password. ID codes are completely randomised. The researchers will select 4 digits to create random codes which can then be assigned to participants, alongside the study start code. All rooms where data is stored will only be accessible via a card key. This will prevent those who are not authorised gaining access into a room where sensitive data (e.g., consent forms) may be. In exceptional circumstances, confidentiality may have to be breached in cases where persons are considered to be at risk or if required by law.

### 9.3) Data analysis and statistics

Data analysis will be conducted at Aberystwyth University. Chemical composition using metabolomics will be conducted at AberInnovation and Aberystwyth University. ELIZAs will be conducted at Aberystwyth University.

## 10) References

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