

Study Protocol and Statistical Analysis Plan

Study title: The Effects of Wild Blueberries on Depressive Symptoms in Young Adults

Acronym: BluMood

Date: 07 April 2022

1. Sample Size Calculation

A mixed-design ANOVA carried out on data from a previous study in adolescents demonstrated a medium effect size (partial $\eta^2 = 0.04$) of a four-week blueberry intervention on depressive symptoms (Fisk, Khalid, Reynolds, & Williams, 2020). An *a priori* power analysis in G*Power with this effect size indicated that a sample of 60 participants would be necessary to achieve a power level of 0.80 at a significance level of 0.05.

2. Recruitment and Screening

Participants will be recruited using opportunity sampling. The study will be advertised through email distribution and with posters across the University of Reading. Before interested participants are enrolled in the trial, we will invite them to the lab for a screening session to determine if they meet the eligibility criteria and give them an opportunity to become familiar with the cognitive task battery so as to reduce the influence of practice effects. Participants will be compensated with £120 (\$156) for completing all phases of the study.

3. Randomization and Blinding Process

A researcher not involved in recruitment or data collection will use blocked randomization with random permuted blocks of two and four individuals to form the allocation list for the two comparison groups. The allocation sequence will be generated using the online randomization tool Sealed Envelope (v1.19.1) and will be concealed to the researchers enrolling and assessing participants. The interventions for chronic consumption will be distributed to participants in opaque, sealed sachets whereas on testing days the intervention drinks will be administered in opaque shaker bottles prepared by research personnel not involved in the conduct of the trial. Thus, both participants and investigators will be blinded to the treatment assignment throughout the study.

4. Testing Protocol

Visit 1

09:00 – participants consume a standard breakfast of oat porridge after an overnight fast

09:40 – participants complete cognitive task + PANAS-X

10:00 – blood sample taken

10:10 – participants consume the drink they have been allocated to (blueberry or placebo)

11:10 – participants complete a battery of questionnaires (e.g. BDI-II, PHQ-9)

11:25 – participants have their heart rate variability measured

12:10 – participants complete cognitive task + PANAS-X

12:25 – participants are given a bag with 41 sachets for the chronic phase of the study

Visit 2

09:00 – participants consume a standard breakfast of oat porridge after an overnight fast

09:30 – participants complete a battery of questionnaires (e.g. BDI-II, PHQ-9)

09:50 – participants have their heart rate variability measured

10:00 – participants complete cognitive tasks + PANAS-X

10:25 – blood sample taken

5. Statistical Analysis Plan

The outcomes will be analyzed using ANCOVAs adjusted for baseline values, sex, and habitual fruit and vegetable consumption as long as the assumptions for conducting the analysis are met.

References

Fisk, J., Khalid, S., Reynolds, S. A., & Williams, C. M. (2020). Effect of 4 weeks daily wild blueberry supplementation on symptoms of depression in adolescents. *Br J Nutr*, 1-8.