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Official title of the study:

Moringa Powder Acceptability Trial Among Healthy Adults

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Statistical Analysis Plan

Version history

Version number	Version date	Prepared/reviewed by	Description of the changes made
1	7/30/23	Caitlin French Susana Matias	Original

Study Aims

Overall objective*: Assess the acceptability and side effects of consuming dried moringa leaf powder (MLP) at 3 different doses (1, 2, or 3 teaspoons) daily for 7 days, among UC Berkeley staff employees.*

Specific Aim 1: Describe the total acceptability score of MLP, and whether it differed by dose group.

Sub-Aim 1.1: Describe the average scores for liking the taste, texture, and appearance of foods after adding MLP, and determine whether they differed by the dose group.

Specific Aim 2: Describe the total number of days MLP was consumed during the 7-day intervention period, and whether it differed by the assigned dose.

Specific Aim 3: Describe the proportion of participants who reported gastrointestinal or other symptoms after consuming MLP and determine whether it differed by dose group.

Secondary Aim 1: Describe the rate of reported willingness to continue consuming MLP and whether it differed by dose group.

Secondary Aim 2: Determine the average number of teaspoons consumed (daily and total) for each dose group, based on available data (daily consumption reports), and whether the proportion of the assigned dose consumed differed by group.

Secondary Aim 3: Determine whether skin carotenoid score (SCS) increased after consuming MLP for one week, and whether the change in SCS differed by dose group.

Study Design and Sample

The study design is an unblinded, randomized controlled trial in which UC Berkeley (non-student) staff members volunteered to be randomly assigned to consume 1, 2, or 3 teaspoons of MLP every day for 7 days. We did not include a control group in order to maximize the sample size for the main outcome of interest, which was the acceptability of consuming moringa.

The analytical samples will differ for each aim based on the outcome data source (daily surveys or final interview), and will include those with available data. See Analysis Principles below for further details on the modified intention-to-treat approach.

Variables

Exposure:

Daily consumption of 1, 2, or 3 teaspoons MLP added to foods or drinks for one week. Total doses of 7, 14, or 21 teaspoons of MLP were provided to participants to take home at the beginning of the study

(day 1), and compliance was measured by questions asked on daily online surveys and the final interview. For main analyses, the exposure will be defined as the intervention group to which the participant was assigned at the beginning of the study.

Outcomes:

- ***Acceptability***. On day 8 of the study, 3 aspects of acceptability (taste, texture, and appearance) of foods or drinks after adding MLP were assessed using a 5-point liking scale, where a reported value of 1 indicated "Dislike it a lot", 2 indicated "Dislike it a little", 3 indicated "Neither like nor dislike", 4 indicated "Like it a little", and 5 indicated "Like it a lot". The text descriptions and numbers were accompanied by emoji faces representing the corresponding level of like or dislike. *Total acceptability scores* will be calculated by summing the 3 *sub-scores*, with a potential range of 3-15 points.
- ***Consumption.*** The *total number of days consuming MLP* during the intervention period will be assessed using the following question asked at the final interview (day 8): "During the past week, how many days did you consume moringa powder?". The *average daily dose* (tsp) consumed will be defined as the mean quantity reported consumed among days where consumption data is available. Among participants with available daily consumption data for ≥ 4 of the 7 intervention days, the total amount (tsp) of MLP consumed during the intervention period will be calculated by multiplying the reported number of days consumed by the average reported amount consumed on consumption days. To measure the degree of compliance, this total will be divided by the total assigned dose and multiplied by 100 to obtain the *percent of the assigned dose consumed*.
- ***Willingness to continue consuming MLP.*** At the final interview (day 8), participants were asked about their *willingness to continue consuming MLP* using the question, "Would you be willing to continue taking the moringa powder?". Response options included "yes", "no", or "not sure".
- ***Symptoms***. Gastrointestinal and other symptoms experienced in the previous 24 hours were assessed on day 1 of the study via the baseline interview, on days 2-7 via the daily survey, and on day 8 via the final interview. Participants were asked whether or not they had experienced diarrhea, bloating, stomach ache, other digestive problems, allergic reactions (e.g. rash, hives, fever, difficulty breathing, low blood pressure, etc.), or other symptoms. The *proportion of participants who reported any symptoms* will be calculated. The *total number of symptoms experienced* will be described as the total number of different symptoms (e.g. diarrhea + bloating = 2 symptoms) reported, 1) on each day, and 2) during the whole intervention period.
- ***Skin carotenoid level***. Skin carotenoid level was assessed on study days 1 and 8 using the Veggie Meter®, a commercially available reflection spectrometry device that outputs a skin carotenoid score (SCS) between 0 and 800, which is correlated with dietary intake of fruits and vegetables. Skin carotenoid levels were measured in triplicate and averaged by the Veggie Meter® software. The *change in SCS* will be calculated by subtracting each participant's baseline SCS from their SCS at the final interview.

Sociodemographic variables

Sociodemographic variables (for reporting sample characteristics) include *age* (years), *gender* (Man, Woman, Non-binary, or Other), *Latinx/Hispanic ethnicity* (Yes or No), and *racial/ethnic identity* (White, Black or African American, American Indian or Alaska Native, Asian, Native Hawaiian or Pacific Islander,

Mixed, or Other). For gender, all respondents reported identifying as a man or a woman, so this variable will be analyzed as binary. For racial/ethnic identity, responses from the two questions will be combined to create a single categorical variable, and categories will be collapsed into fewer categories if needed to allow for analysis.

Analysis principles

- Outcomes will be analyzed based on a modified intention-to-treat approach, in which 1) participants with missing outcome data will be excluded (i.e. no imputation will be done), and 2) one participant who was erroneously assigned to a different group from what the randomization order prescribed will be analyzed according to the dose they actually received and were instructed to consume.
- Distributions of variables will be described using frequencies and percentages, in the case of categorical variables, mean (SD) or median (IQR) for continuous variables, and one or both of these for ordinal or count variables.
- Classic ANOVA, Welch's ANOVA, or Kruskal-Wallis tests to detect group differences of ordinal, count and continuous variables will be selected based on whether the following assumptions are met:
    - The distribution within each group is approximately normal based on visual inspection of the data and normal probability plots (Classic ANOVA, Welch's ANOVA).
    - Group variances are approximately equal, i.e., the ratio of the largest to the smallest variance is ≤ 1.5[1,2] (Classic ANOVA).
- Chi Square or Fisher's Exact test will be used to analyze differences by intervention group for categorical variables.
- Significance level will be set at 0.05. Hypothesis testing results will be interpreted in conjunction with effect sizes and overall trends in the data.
- *Missing data*: For outcomes measured only at the final survey, observations from participants who did not complete the final interview will be excluded from analysis (e.g. Aim 1). For other outcomes, including those measured by daily surveys, available case analysis (e.g. cases with data at each daily survey) will be conducted.
- *Outliers*: Distributions of continuous variables will be visually inspected for outliers, and potential outliers will be investigated further for input errors or other explanations. If no rationale for removing the observation is identified, non-parametric rank-based methods will be considered and/or sensitivity analyses will be conducted to determine the effect of removing the outlier on the results.

Baseline participant characteristics

Descriptive summary statistics for sociodemographic and other variables will be reported overall and by intervention group. Age and baseline skin carotenoid scores will be summarized as mean (SD) or median (IQR). Gender identity, racial/ethnic identity, and gastrointestinal and other symptoms (any, as well as individual symptoms) reported by participants in the last 24 hours at baseline will be reported as frequency (%).

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Primary Analyses

Aim 1 (Acceptability): Distributions of total acceptability scores and sub-scales will be presented as mean (SD) or median (IQR). We will investigate average differences between the 3 intervention groups in total acceptability scores and sub-scores using one-way ANOVA, Welch's ANOVA or Kruskal Wallis tests.

Aim 2 (Consumption): We will present the proportion of participants who reported consuming MLP for the full 7 days vs. less, as well as the proportion of participants reporting each frequency of consumption (e.g. 5 days, 6 days, etc.) and/or the mean (SD) or median (IQR) number of days consumed. All descriptive statistics will be reported overall and by intervention group. To investigate whether the number of days differed by intervention group, we will conduct Kruskal-Wallis tests.

Aim 3 (Symptoms): The proportion of participants reporting symptoms (any, as well as individual symptoms) during the intervention period will be reported overall (whole intervention period) and by study day for each group. The total number of symptoms reported will be presented in a similar manner. In addition, line graphs will be generated to visualize individual participant trajectories in terms of the number of symptoms reported each day and response/non-response to the daily surveys, and will be color-coded by assigned dose to visualize trends by group. Chi-square or Fisher's Exact test will be used to test whether the frequency of reporting any symptoms differed by assigned dose. ANOVA, Welch's ANOVA or Kruskal-Wallis tests will be used to test whether the total number of reported symptoms differed by dose group.

Secondary Analyses

Secondary Aim 1 (willingness to consume): We will report the proportion of participants who reported that they were willing to continue taking the MLP overall and by dose, and investigate whether the distribution of responses differed by dose group, using Chi-square tests if sample sizes allow.

Secondary Aim 2 (consumption amount): Average daily dose consumed on consumption days, total MLP consumption (tsp) during the intervention period, and the proportion consumed of the assigned dose will be presented as mean (SD) or median (IQR). All descriptive statistics will be reported overall and by intervention group. To investigate whether the proportion of assigned dose consumed differed by dose group, we will conduct one-way ANOVA, Welch's ANOVA or Kruskal-Wallis tests. Line graphs will be generated to visualize individual participant trajectories of the daily amount consumed and response/non-response to the daily surveys.

Secondary Aim 3 (skin carotenoid score): We will report baseline and post-intervention distributions of SCS by dose group as mean (SD) or median (IQR). To determine whether SCS changed after consuming MLP for one week, we will present the distribution of pre-post-intervention changes in SCS as mean (SD) or median (IQR), by dose group. To investigate whether the change in SCS differed by assigned dose, we will perform ANOVA, Welch's ANOVA or Kruskal-Wallis tests of the change in SCS. As a sensitivity analysis, if the model assumptions for linear regression can be met, we will model change in SCS by regressing endline SCS on intervention group, while adjusting for baseline SCS in the following linear regression model: *Endline SCS ~ Dose group + Baseline SCS*.

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Reference

1. Lowry R. One-Way Analysis of Variance for Independent Samples. Part 1. In:2002.
2. Morgan CJ. Use of proper statistical techniques for research studies with small samples. *American Journal of Physiology-Lung Cellular and Molecular Physiology.* 2017;313(5):L873-L877.

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Appendix. Description of Planned Qualitative Analyses

In this small pilot study, quantitative results will be interpreted together with the following qualitative analyses of free-response interview questions:

1. **Qualitative Aim 1**. Describe how participants consumed the moringa powder they received and whether there were patterns in response by the dose consumed.
    a. **Data**: Mode of consumption was assessed at the final interview using the multiple-choice question, "How did you mostly consume the moringa powder?". Answer options included "mixed with cold food", "mixed with hot food", "sprinkled over cold food", "sprinkled over hot food", "mixed with cold drinks/smoothies", "mixed with hot drinks", "mixed with water" or other.
    b. **Approach**: Open-ended responses will be coded. Responses to this question will be summarized using frequencies.
2. **Qualitative Aim 2**. Describe what participants liked and disliked most about the moringa powder they received and whether there were patterns in responses by the dose consumed.
    a. **Data**: In addition to quantitative scales, acceptability was assessed using 2 open-ended questions, which included "What did you like the most about the moringa powder you received?" and "What didn't you like about the moringa powder?". Responses were recorded (typed in) verbatim or near verbatim by the interviewer.
    b. **Approach**: To qualitatively describe trends in what participants liked most or disliked about MLP, qualitative thematic analysis will be conducted. Open-ended responses will be coded using pre-specified and emergent themes. Visual aids (e.g. word bubbles, hierarchical graphics, etc.) and tables of themes with the frequency reported will be presented.
3. **Qualitative Aim 3**: Qualitatively describe whether acceptability of MLP differed by the mode of consumption (e.g., in smoothies, sprinkled over food, etc.).
    a. **Data**: Total acceptability scores, responses from open-ended questions in which mode of consumption was discussed
    b. **Approach**: To explore whether acceptability of MLP might differ by the mode of consumption, the mean (SD) of acceptability scores will be calculated by reported mode of consumption, and relevant responses to open-ended questions will be summarized.
4. **Qualitative Aim 4**: Qualitatively report reasons for non-compliance.
    a. **Data**: If participants reported not consuming the MLP for the whole 7 days, they were asked to give their reason for not consuming MLP.
    b. **Approach**: Responses to this question will be summarized within the text.