United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034009

Project Title

PARTNERSHIP: Cardiometabolic-Protective And Gut Microbiota Modulatory Effects Of Red Cabbage Microgreens: A Plants To Health Approach

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
Funding Source NIFA Non Formula	**Grant Year** 2026
Grant No. 2026-67017-45557	**Cumulative Award Amt.** $775,934.00
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Program Code [A1343] Food and Human Health	

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Performing Department

Department of Health, Nutrition and Food Sciences

Non Technical Summary

Microgreens are young leafy greens of vegetables, herbs, grains, and flowers with potential to sustainably diversify food systems and support human health. Their diverse flavors, textures, and colors,nutritional quality, and other factors have have led to increased industry and consumer awareness and use. Research is limited but supports their nutritional quality, likeability by consumers, and potential human health benefits. Research is needed to advance our understanding from nutritional and human health perspectives as their health effects in humans are unknown. In particular, research that can optimize agricultural production of high nutritional quality microgreens, evaluate their consumer acceptabilityand human health is warranted. Red cabbage microgreens are promising due to their nutritional quality, consumer acceptability, feasibility for daily consumption, and potential cardiometabolic health and gut microbiome modulatory benefits. The overall objective of this project is to optimize the nutritional quality of red

cabbage microgreens through agricultural practices to improveheart and gut health benefits formiddle-aged/older adults. We beleive that we can optimize red cabbage microgreen nutritional quality through the lighting environment to support human healthwhile maintaining microgreen quality characteristics and consumer acceptability necessary for agricultural and food industries, and the consumer. The proposed studies will improve our understanding of the impact of red cabbage mirogreens on human health, which can be utilized in US agricultural and food systems to improve food quality to better promote human health.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	70%
Applied	20%
Developmental	10%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
701	1430	1010	20%
701	1430	3090	20%
203	1430	1060	10%
702	1430	1010	40%
204	1430	3090	10%

Knowledge Area

[701] Nutrient Composition of Food

[203] Plant Biological Efficiency and Abiotic Stresses Affecting Plants

[702] Requirements and Function of Nutrients and Other Food Components

[204] Plant Product Quality and Utility (Preharvest)

Subject Of Investigation

[1430] Greens and leafy vegetables (includes endive, lettuce, spinach, turnip-greens, celery,rhubarb, parsley, asparagus)

Field Of Science

[1010] Nutrition and metabolism

[3090] Sensory science (human senses)

[1060] Biology (whole systems)

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Keywords

Phytochemicals, bioavailability, biofortification, nutritional quality, Red cabbage microgreens

Goals / Objectives

Our overall objective is to optimize red cabbage microgreen nutritional quality with an emphasis on selecting health-promoting phytochemicals (e.g., anthocyanins, glucosinolates) through manipulation of the light and temperature and to establish the feasibility and efficacy of implementing daily microgreen consumption to favorably modulate cardiometabolic health and gut microbiome in a population at high-risk for endothelial dysfunction and CVD. We propose the following specific aims, using a translational approach that spans plants, cells, and humans to test the central hypothesis that red cabbage microgreen nutritional quality can be optimized to improve human cardiometabolic health and favorably modulate gut microbiome while maintaining quality characteristics and consumer acceptability. Aim 1: To optimize red cabbage microgreen nutritional quality while maintaining microgreen morphological quality, intestinal nutrient and phytochemical uptake, and consumer acceptability. Red cabbage microgreens and mature red cabbage will be produced using a standard greenhouse environment or a multi-layered production system using sole-source light-emitting diode (LED) lighting. Sole-source LED lighting and temperature will be used as a means of biofortification. Specifically, the impact of far-red light (700-780 nm) and reduced air temperature on microgreen quality will be investigated. Conventionally (greenhouse) grown and biofortified red cabbage microgreens, and mature red cabbage will be evaluated for their nutritional quality, potential bioavailability of select nutrients and phytochemicals, and consumer acceptability.Outcome measures will include: 1) characterization of microgreen nutritional quality through analysis of micronutrients and phytochemicals by mass spectrometry to determine types and quantities across growing conditions and relative to mature red cabbage; 2) evaluation of intestinal cell uptake of select micronutrients and phytochemicals of red cabbage microgreens grown under different conditions and compared to mature red cabbage; 3) evaluation of plant morphological quality attributes; and 4) assessment of consumer acceptability of biofortified red cabbage microgreens relative to conventionally (greenhouse) grown red cabbage microgreens and mature red cabbage using a consumer panel (n=100). We hypothesize that 1) target phytochemical quantities (anthocyanins and glucosinolates) can be increased in red cabbage microgreens relative to mature red cabbage through optimization of the controlled environment; 2) increased nutritional quality will be paralleled by no changes or increases in intestinal uptake of the target phytochemicals and nutrients for optimization; and 3) there will be no adverse effects on microgreen yield, morphological quality, or consumer acceptability.Aim 2: To evaluate the cardiometabolic modulatory effects of daily red cabbage microgreen consumption and gain mechanistic insight involving the gut microbiome and (poly)phenol metabolites in middle-aged/older adults (MA/O) with above-normal BP. A randomized, controlled, parallel-arm clinical trial will be performed for 8 weeks in adults aged 45-75 years with above-normal BP (initial n=105; n=35/group) to evaluate consumption of 1) one cup/day red cabbage microgreens (low dose), 2) two cups/day red cabbage microgreens (high dose), or 3) no intervention (control). This population is at high risk for endothelial dysfunction (central to CVD). Primary outcome measures will include assessment of physiological measures of endothelial function, arterial stiffness, BP, and other cardiometabolic biomarkers. Secondary outcomes will include taxonomic and functional characterization of the gut microbiome, targeted plasma and urine (poly)phenol metabolite analysis by mass spectrometry to gain mechanistic insight and evaluate determinants of inter-individual variability in physiological responses. These will help identify underlying mechanisms and individualized responses to red cabbage microgreen intervention and allow for statistical evaluation of the relationship between measures of cardiometabolic health, gut microbiota, and phytochemical metabolites in biological samples. We hypothesize that 1) red cabbage microgreens will dose-dependently improve cardiometabolic health and 2) that cardiometabolic responses and phytochemical exposure will be associated with gut microbiome composition and function.

Project Methods

Aim 1: To optimize red cabbage microgreen nutritional quality while maintaining microgreen morphological quality, intestinal nutrient and phytochemical uptake, and consumer acceptability.Microgreen Production and Quality. Red

cabbage microgreens will be placed under one of three light treatments with a 16-h photoperiod varying in photosynthetic photon flux densityand supplemental far-red light (700-780 nm). Temperature treatments will be established in separate walk-in growth chambers by maintaining constant air temperatures of 18, 21, or 24 °C. After two weeks, microgreens will be harvested, and data collected on yield, morphological quality and micronutrient and phytochemical concentrations.Mature Red Cabbage Production. Brassica oleracea var. capitata seeds will be sown into 200-cell rockwool cubes (A-OK Starter Plugs; Grodan). After approximately 2 weeks, rooted plugs will be transplanted into nutrient film technique (NFT) hydroponic systems (NFT 8-25; CropKing) located in actively cooled greenhouse with VersiSTEP environmental control systems. Plants will be provided a recirculating nutrient solution of 150 ppm N derived from two water-soluble fertilizers, Jack's Hydroponic 15-12-26 and calcium nitrate. Nutrient reservoirs will be monitored daily to maintain target electrical conductivity and pH values.Metabolomics and Ionomics Analysis of Plants. We will utilize liquid chromatography mass spectrometry (LC-MS) methods and data analysis pipelines developed as part of the Periodic Table of Food Initiative (PTFI).In Vitro Assessment of Intestinal Uptake. Caco-2 cells will be cultured and grown into a confluent, polarized monolayer. Fresh microgreens will be digested following the INFOGEST simulated digestion model as described.The MTT assay will be used to measure cell viability and optimize experimental conditions prior to treating Caco-2 cells; The collected digesta will be added to theapical compartment of each transwell system and incubated. A single aliquot of the sample added to the apical chamber will be stored for analysis (T=0), and aliquots will be collected from the basolateral chamber after 30, 60, 120 and 240 min. Consumer Acceptability and Sensory Perceptions. 100 consumersaged 45-75 years will be recruited that have no aversions to leafy green vegetables, non-smokers, and not taking antibiotics or being treated for colds, flu, allergies, or nausea. A consumer acceptability and sensory perception study will be performed with 5 g samples of red cabbage microgreens provided in white containers and labeled with a 3-digit random number for blinding. A total of 15 sessions will be conducted over a 3-day period with no more than 8 participants/session in a quiet environment and separated for privacy. Participants will be provided with written and verbal instructions, and provided ballots.Aim 2: To evaluate the cardiometabolic modulatory effects of daily red cabbage microgreen consumption and gain mechanistic insight involving the gut microbiome and (poly)phenol metabolites in MA/O adults with above-normal BP.Experimental Design. 105 adults aged 45-75 years with above-normal BPwill be enrolledin a randomized, open-label, controlled, parallel-arm clinical trial. Inclusion/exclusion criteria are in Table 1. Table 1. Inclusion and Exclusion Criteria.Inclusion Criteria: Aged 45-75 yearsFemales should be postmenopausal (?1 years; natural or surgical menopause; confirmed by measurement of estradiol < 30 pg/mL and FSH ? 30 mIU/mL)Elevated systolic BP or HTN (i.e., resting seated brachial systolic BP > 120 mmHg)Willing to be randomly assigned to an interventionWilling to maintain usual diet, physical activity, and medication useProvide informed consentExclusion Criteria:Systolic BP < 120 or ? 180 mmHg or diastolic BP > 120 mmHgTaking > 1 antihypertensive medication or taking the medication for < 3 monthsBody mass index < 18.5 or > 40 kg/m2Cancer, CVD, diabetes, or GI, kidney, liver, and/or pancreatic diseaseTriglycerides >350 mg /dL, low-density lipoprotein cholesterol ? 190 mg/dL, or taking lipid-lowering medication in 3 months prior to studyHbA1c > 6.4% or taking glucose control medicationHormone therapy use in 6 months prior to studyUse of warfarin in 3 months prior to studyTobacco/nicotine use in 12 months prior to studyHeavy or binge alcohol drinkersContraindication to treatments or proceduresAn overview of the data being collected at 4 study visits is listed below:Visit 1 (Screening): Informed consent and onsite screeningBP and blood biomarker assessment, medical/health history, anthropometrics, and evaluation of inclusion/exclusion criteriaVisit 2 (Week 0): Baseline assessments:BP and vascular function assessmentBlood, urine, and fecal sample collectionFood frequency questionnaire (FFQ), diet record, physical activity recall (PAR), anthropometrics, and body compositionTreatment distributionVisit 3 (Week 4): Midpoint assessmentsTreatment compliance check and distributionBP, blood, urine, and fecal sample collectionFFQ, Diet record, PAR, and anthropometricsVisit 4 (Week 8): Endpoint assessmentsTreatment compliance checkBP and vascular function assessmentBlood, urine, and fecal sample collectionFFQ, diet record, PAR, anthropometrics, and body compositionRandomization, Treatments, Dietary Inclusion, and Compliance. Participants will be randomly assigned treatment groups (initial n = 35/group): 1) 1 cup/day red cabbage microgreens (low dose; 15 g fresh weight), 2) 2 cups/day red cabbage microgreens (high dose; 30 g fresh weight), or 3) no intervention (control).Preliminary DIM Compliance Biomarker Study: A total of 5 individuals (same inclusion/exclusion criteria as main RCT) will consume 1 or 2 cups/day of microgreens in the presence of the investigators for 3 days on 2 separate occasions separated by 1

week to determine the targeted urinary amount range (total urinary DIM excreted in 24 h) to define compliance in the main RCT. We will collect 24 h urine at baseline and after 3 days post-consumption, determine within and between variability and use the results to calculate compliance thresholds.?Sample Size and Statistical Analyses. SAS V9.4 will be used. Data will be measured on a continuous scale and evaluated for normality prior to performing a linear regression analysis with repeated measures over time. 'Intervention' and 'time' will be included in the model as fixed effects with an interaction term between the variables. Potential confounders will be included in the model to adjust for their effects.Statistical significance will be set at $P<0.05$. Significantly changing microbial populations and metabolites will be identified using a mixed model ANOVA including factors 'treatment' and 'time' as fixed factors, and 'participant' as a random factor to account for the repeated measures design. Tukey's HSD post-hoc testing with false discovery correction will be used to determine microbial populations and metabolites that differ at the later sampling point between groups. Significantly altered microbial populations and metabolites will then be modeled independently in linear regression models within each group to assess the extent to which they predict outcomes. For the microbial 16s rRNA sequence data, multivariate statistical analyses will be conducted using QIIME2 and MicrobiomeAnalyst. Data will be cleaned and curated using the DADA2 pipeline and non-parametric statistical tests will be used for determining differences among treatment groups. Alpha diversity measures will include CHAO, Shannon, Simpson, and Faith's. Bray-Curtis and Unifrac distances will be used as input for PCA. Negative binomial GLM (EdgeR) and LeFSe will be used to detect treatment-associated biomarkers.