Cover Page for Protocol

NCT number:	NCT05773794
Official title of study:	Scavenging of reactive carbonyl species by dietary flavonoids from Soymilk, green tea, and blueberry in humans
Document date:	3/15/2023
Brief Summary:	This is an interventional study to investigate the formation and pharmacokinetics of reactive carbonyl species adducts of dietary polyphenols (soymilk, blueberry and green tea) in humans after a single dose of dietary flavonoids.

Background

Carbonyl stress is the abnormal accumulation of carbonyl metabolites, such as methylglyoxal and acrolein, leading to increased modification of protein and DNA and contributing to cell and tissue dysfunction in aging and many chronic diseases. Dietary flavonoids have been reported to have the capacity to scavenging of these reactive carbonyl species in vitro and in mice. However, this effect has not been demonstrated in humans.

Study Objectives

The goal of this study is to determine the formation and kinetics of reactive carbonyl adducts of dietary flavonoids (soymilk, blueberry and green tea) in humans after a single dose of the dietary flavonoids. We speculate that the dietary flavonoids (soymilk, blueberry and green tea) can react with endogenous reactive carbonyls species to form reactive carbonyl adducts in the body, eventually alleviating the damaging effects of reactive carbonyls species on human health. Study on the formation and kinetics of reactive carbonyl adducts of dietary flavonoids is also expected to be able to provide substantial evidence for the health effects of regular dietary flavonoids (soymilk, blueberry and green tea) consumption in epidemiological studies.

Study Design

This study follows a **randomized, crossover design**, where participants will consume each intervention (soymilk, blueberry, and green tea) in separate study periods with a washout phase in between.

- **Study Duration:** 17 days
- **Number of Participants:** 16 healthy adults for each intervention
- **Intervention Groups:**
    - Dietary Flavonoids (Soymilk) Consumption and Placebo
    - Dietary Flavonoids (Blueberry) Consumption and Placebo
    - Dietary Flavonoids (Green Tea) Consumption and Placebo

Study Methods- Participant Selection

Minimum Age: 25 Years
Maximum Age: 70 Years
Sex: All
Criteria:

Inclusion Criteria:

1. Age 25-70 years
2. BMI between 18 and 30
3. Have no known allergy to soy milk
4. Be not taking antibiotics for six months
5. Be not currently taking medication
    a. No taking any prescription drugs
    b. If supplements were taken, flavonoid-enriched supplements (see list below) should be avoided.
    Major brands Notes: All flavonoid-enriched supplements should be avoided. Soy Isoflavones/ tea/blueberry Lipo-Flavonoid Citrus Bioflavonoids Complex Super Flavonoids Super Antioxidants Quercetin Supplement Essential-C and flavonoids Milk Thistle Other flavonoids supplements, including but not limited: Luteolin, Rutin, etc
    c. No any drugs or supplements within three weeks of the experiment
6. Be nonsmoking
7. Have no alcoholic intoxication

a. No alcoholic addiction
b. < 3-4 drinks per week (less than 2 glasses (300 mL) per drink)
c. No alcohol within 3 three weeks of the experiment

8. Have no extended exposure to industrial wastes

Exclusion Criteria:

1. Disease: gout, heart disease, peripheral vascular disease, degenerative kidney, degenerative liver, diabetes, GI disorders, or endocrine disorders
2. Cancer patients
    a. Currently diagnosed cancer patients will be excluded;
    b. Medication free for >2 years could be considered

Study Methods- Procedures

1. Dietary Flavonoids (Soymilk) Consumption and Placebo

Week 1 (Wash-in Period: Days 1–7)

Sixteen (16) healthy volunteers were recruited and instructed to avoid consuming soymilk, green tea, and blueberry products starting one week before the intervention and continuing throughout the entire three-week study period.

Week 2 (First Intervention: Day 8)

Participants were randomized into two groups:

- **Soymilk-first group**: Consumed a breakfast including 460 mL of soymilk in a single dose.
- **Control-first group**: Consumed a breakfast including 460 mL of milk in a single dose.

Biological sample collection:

- Urine and blood samples were collected at multiple time points over a 24-hour period.
- Fecal samples were collected at different time points over a 48-hour period.

Participants continued to avoid consuming soymilk, green tea, and blueberry products.

Week 3 (Cross-over: Day 15)

Participants switched interventions:

- Those who previously consumed soymilk in Week 2 now consumed milk.
- Those who previously consumed milk in Week 2 now consumed soymilk.

Biological sample collection:

- Urine and blood samples were collected at multiple time points over a 24-hour period.
- Fecal samples were collected at different time points over a 48-hour period.

Participants continued to avoid consuming soymilk, green tea, and blueberry products until Day 17.

2. Dietary Flavonoids (Blueberry) Consumption and Placebo

Week 1 (Wash-in Period: Days 1–7)

Sixteen (16) healthy volunteers were recruited and instructed to avoid consuming soymilk, green tea, and blueberry products starting one week before the intervention and continuing throughout the entire three-week study period.

Week 2 (First Intervention: Day 8)

Participants were randomized into two groups:

- **Blueberry-first group**: Consumed a breakfast including 460 mL of blueberry water in a single dose.
- **Control-first group**: Consumed a breakfast including 460 mL of placebo sweet water in a single dose.

Biological sample collection:

- Urine and blood samples were collected at multiple time points over a 24-hour period.
- Fecal samples were collected at different time points over a 48-hour period.

Participants continued to avoid consuming soymilk, green tea, and blueberry products.

Week 3 (Cross-over: Day 15)

Participants switched interventions:

- Those who previously consumed blueberry water in Week 2 now consumed placebo sweet water.
- Those who previously consumed placebo sweet water in Week 2 now consumed blueberry water.

Biological sample collection:

- Urine and blood samples were collected at multiple time points over a 24-hour period.
- Fecal samples were collected at different time points over a 48-hour period.

Participants continued to avoid consuming soymilk, green tea, and blueberry products until Day 17.

3. Dietary Flavonoids (Green Tea) Consumption and Placebo

Week 1 (Wash-in Period: Days 1–7)

Sixteen (16) healthy volunteers were recruited and instructed to avoid consuming soymilk, green tea, and blueberry products starting one week before the intervention and continuing throughout the entire three-week study period.

Week 2 (First Intervention: Day 8)

Participants were randomized into two groups:

- **Green tea-first group**: Consumed a breakfast including 460 mL of green tea in a single dose.

- **Control-first group**: Consumed a breakfast including 460 mL of water in a single dose.

Biological sample collection:

- Urine and blood samples were collected at multiple time points over a 24-hour period.
- Fecal samples were collected at different time points over a 48-hour period.

Participants continued to avoid consuming soymilk, green tea, and blueberry products.

Week 3 (Cross-over: Day 15)

Participants switched interventions:

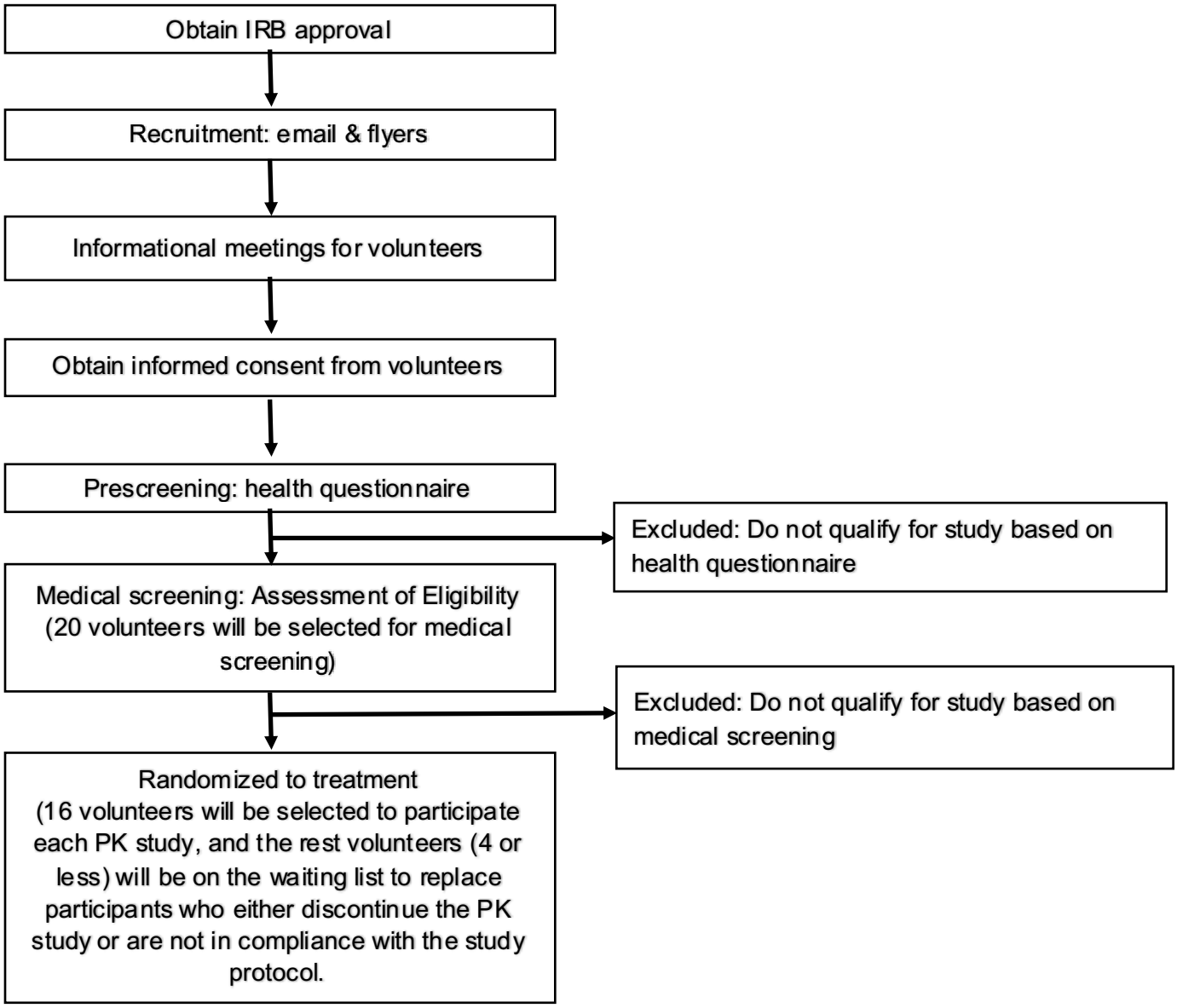
- Those who previously consumed green tea in Week 2 now consumed water.
- Those who previously consumed water in Week 2 now consumed green tea.

Biological sample collection:

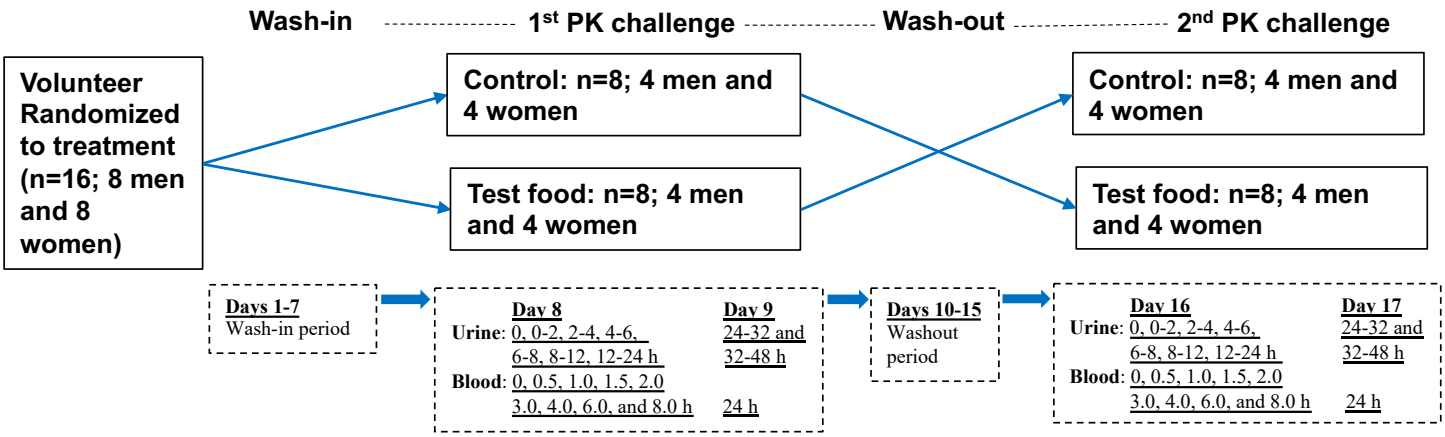
- Urine and blood samples were collected at multiple time points over a 24-hour period.
- Fecal samples were collected at different time points over a 48-hour period.

Participants continued to avoid consuming soymilk, green tea, and blueberry products until Day 17.

Scheme 1. Recruitment, screening, and enrollment for each PK study



Scheme 2. Experimental design and sample collections of the acute PK study with a crossover design



Calendar for Fecal Sample Collection_Human Study_2023						
Day 0 (Consenting) ○ Pick up cooler and fecal collector;	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Day 7 ○ Collect baseline (0 h) fecal samples;	**Day 8** (Control) ○ Bring baseline fecal sample to HRC; ○ Pick up another cooler and fecal collector; ○ Collect 24-h fecal samples;	**Day 9** ○ Bring 24-h fecal sample to HRC; ○ Pick up another cooler and fecal collector; ○ Collect 48-h fecal samples;	**Day 10** ○ Bring 48-h fecal sample to HRC; ○ Pick up another cooler and fecal collector;	**Day 11**	**Day 12**	**Day 13**
Day 14 ○ Collect baseline (0 h) fecal samples;	**Day 15** (Treatment) ○ Bring baseline fecal sample to HRC; ○ Pick up another cooler and fecal collector; ○ Collect 24-h fecal samples;	**Day 16** ○ Bring 24-h fecal sample to HRC; ○ Pick up another cooler and fecal collector; ○ Collect 48-h fecal samples;	**Day 17** ○ Bring 48-h fecal sample to HRC;	**Day 18**	**Day 19**	**Day 20**

Statistical Analysis Plan

The primary objective of this study is to evaluate whether dietary flavonoids from soy milk, blueberries and green tea can trap reactive carbonyl species in humans by detecting the formation of carbonyl adducts of these flavonoids.

Statistical Analysis: ANOVA with repeated measures and post hoc comparisons will be conducted to identify metabolites that show significant differences across various time points or treatments.

Power Analysis: A sample size of n=16 subjects per group will provide 80% power to detect metabolites with an effect size of 0.809, which is sufficient to assess the expected trapping effects of dietary flavonoids.

INFORMED CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Adult Participants

North Carolina A&T State University
Consent Form Version Date: November 22, 2022
IRB Study #22-0093
Study Title: Scavenging of reactive carbonyl species by dietary flavonoids in humans
Principal Investigator: Shengmin Sang
Principal Investigator Department: Center for Excellence in Post-Harvest Technologies (CEPHT)
Principal Investigator Phone Number: 704-250-5710
Principal Investigator Email Address: ssang@ncat.edu
Funding Source and /or Sponsor: NIH R01
Study Contact Telephone Number: 704-250-5083, 704-250-5711
Study Contact Email: hrc_studies@unc.edu, sanglabncat@gmail.com

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What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary.
You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in the studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or North Carolina A&T State University. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this content form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is to determine whether drinking soy milk can lower the harmful reactive carbonyl species (RCS) in humans. RCS is derived from the oxidation of carbohydrates, lipids, and amino acids in the body. Abnormal accumulation of RCS contributes to cell and tissue dysfunction in aging and many chronic diseases. Soy isoflavones have been reported to have the capacity to scavenging of these reactive carbonyl species *in vitro* and in mice. However, this effect has not been demonstrated in humans. Therefore, the objective of this study is to determine if isoflavones from drinking soy milk will scavenge RCS and lower the levels of RCS in humans.

Are you qualified to be in this study?

Inclusion Criteria

1. Age 25-70 years
2. BMI between 18 and 30
3. Have no known allergy to soy milk
4. Be not taking antibiotics for six months
5. Be not currently taking medication
   a. No taking any prescription drugs

b. If supplements were taken, flavonoid-enriched supplements (see list below) should be avoided.

Major brands	Notes
Soy Isoflavones	All flavonoid-enriched supplements should be avoided.
Lipo-Flavonoid	
Citrus Bioflavonoids Complex	
Super Flavonoids	
Super Antioxidants	
Quercetin Supplement	
Essential-C and flavonoids	
Milk Thistle	
Other flavonoids supplements, including but not limited: Luteolin, Rutin, etc	

c. No any drugs or supplements within three weeks of the experiment

6. Be nonsmoking
7. Have no alcoholic intoxication
   a. No alcoholic addiction
   b. < 3-4 drinks per week (less than 2 glasses (300 mL) per drink)
   c. No alcohol within 3 three weeks of the experiment
8. Have no extended exposure to industrial wastes

Are there any reasons you should not be in this study?

Exclusion Criteria

1. Disease: gout, heart disease, peripheral vascular disease, degenerative kidney, degenerative liver, diabetes, GI disorders, or endocrine disorders
2. Cancer patients
   a. Currently diagnosed cancer patients will be excluded;
   b. Medication free for >2 years could be considered;

How many people will take part in this study?

There will be 16 people (8 males and 8 females) in this research study.

How long will your part in this study last?

You will be participating in a 3-week study. There will be 1 week of pre-washout period (from Day 1-7) and two weeks (Day 8-17) for sample collection. On Day 0, you need to come to the research facility and sign the Consent form and pick up the fecal collection kit. You will stay at the research facility for two entire days from 7:30 am to 4:30 pm on Day 8 and Day 15, respectively. On these two days, breakfast, lunch, and dinner will be provided. On Days 9, 10, 16, and 17, you need to drop off the urine and fecal samples at the Research facility.

What is your food restriction during this study?

First, make sure you do not have allergic reactions to soy milk consumption or you will be asked to quit.
You will be asked not to drink any soy milk products and not take any medicines and supplements starting 1 week before the study and continuing throughout the entire study for a total of 3 weeks. In addition, you should follow the Food Restriction List below to avoid flavonoid-rich foods during the 3 weeks. It's not allowed to eat restricted foods (**in red**). Also, you need to reduce to consume the limited foods (**in yellow, no more than 1 serving per day**). But you can eat the allowed foods (**in green**) as usual. We provide breakfast, lunch, and dinner for two days (days 8 and 15) when you stay at the research facility for blood drawing and urine sample collection.

1. **Restricted food**

		Notes
Soybeans and soy products	Green soybeans	Food Restricted
	Soy products(flour, fiber, meals, etc)	
	Tofu	
	Soy milk	
	Soy cheese	
	Fava beans	
Fruits	Blackberries	Food Restricted
	Cranberries	
	Blueberries	
	Raspberries	
	Strawberries	
	Grapes	
	Oranges	
	Cherries	
	Apples	
	Plum	
Vegetables	Parsley	Food restricted
	Cabbages	
Fruit juice	Orange juice	Food Restricted
	Blackberry juice	
	Cranberry juice	
	Strawberry juice	
	Grapefruit juice	
	Lemon juice	
	Lime juice	
	V8	
Beverage	Tea	Food Restricted

Foods that should be restricted are included but not limited to this table. Please be notified that all foods containing high levels of polyphenols/flavonoids (>100 mg per 100 g, fresh weight) should be avoided by participants.

2. **Limited food**

		Notes: no more than 1 serving per day
Vegetables	Eggplants	Food limited
	Kales	
	Asparagus	
	Spinach	
	Onions	
	Purple sweet potatoes	
Nuts	Almonds	Food limited

3. **Allowed food**

		Notes
Eggs, meat, and dairy	Eggs	Food allowed
	Chicken meat	
	Beef and pork	

	Whole milk	
Cereal and cereal products	**Corn, barley, wheat**	**Food allowed (Refined-grain products are recommended.)**
	Pizza	
	Noodles, pasta,	
	Brown rice	
	Spaghetti	
Fruits	**Avocados**	**Food allowed**
	Melons	
	Peaches	
	Pears	
	Bananas	
Fruit juice	**Crowberry juice**	**Food allowed**
	Grape juice	
Vegetables	**Green beans**	**Food allowed**
	Broccoli	
	Peas	
	Potatoes	
	Sweet potatoes (except purple sweet potatoes)	
	Corns	
	Celery	
	Leeks	
	Lettuce	
	Cucumber	
	Tomatoes	
Beverage	**Coffee**	**Food allowed**
Nuts	**Various nuts (except almonds)**	**Food allowed**

What will happen if you take part in the study?

All below mentioned procedures are a requirement for participation in the study. Regarding the study visit survey, you may choose not to answer a question for any reason. This is a crossover study. You will receive around 460 ml of soy milk or regular milk with breakfast on Day 8 after 1 week of wash-out. Then on Day 15, you will receive regular milk or soy milk.

- Screen visit (Day 0): If you meet the initial inclusion criteria, you will report to the UNC Nutrition Research Institute in Kannapolis to meet with the study coordinator to determine study eligibility on day 0. After consenting, you will provide your age, height, and weight, then receive the food diary form to record what you eat during the entire study and a fecal sample collection kit.
- First visit (Day 8, whole day): After the wash-out from day 1 to 7, you will receive breakfast (sausage, egg, and cheese biscuit, or other breakfast made in the UNC-HRC kitchen) with a glass of unsweetened soy milk (around 460 ml) on day 8. You have 20 min to finish breakfast. Blood samples will be drawn through an indwelling needle at 0 (before breakfast), 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, and 8.0 h after breakfast (10 mL at each timepoint) by the UNC-HRC. An indwelling needle here refers to a peripheral venous catheter (PVC) that is placed into a peripheral vein on your arm (no needle left in the vein). Upon insertion, the line can be used to draw blood at different time points. Lunch will be provided by the UNC-HRC kitchen, and dinner will be prepared and can be taken back home.

  Also, the urine samples will be collected using plastic containers (provided by the researcher) at baseline (before breakfast), 0-2 h, 2-4 h, 4-6 h, and 6-8 h after breakfast during the stay at the Human Research Core at the UNC Nutrition Research Institute (UNC-HRC), and between 8-12 h and 12-24 h at home. Fecal samples

will be collected at your convenience using the fecal collection kit (provided by the researcher) at baseline, 24 h (Day 9), and 48 h (Day 10).

- Second visit (Day 9): The 24-h blood sample will be collected the next morning before breakfast (day 9). Also, you need to drop off the urine samples of 8-12 h and 12-24 h collected at home, and the fecal sample of 24 h (Day 9). **You will get the first payment after that**.
- Third visit (Day 10): You just need to drop off the fecal sample of 48 h (day 10) at the research facility.
- Fourth visit (Day 15, whole day): On day 15, you will receive breakfast (the same size as the First visit) with regular milk (around 460 ml). The procedures of sample collection (blood, urine, and fecal samples) are the same as on Day 8.
- Fifth visit (Day 16): The 24-h blood sample will be collected the next morning before breakfast (day 16). Also, you need to drop off the urine samples of 8-12 h and 12-24 h collected at home, and the fecal sample of 24 h (day 16). **You will get the second payment after that.**
- Sixth visit (Day 17): You just need to drop off the fecal sample of 48 h (day 17) at the research facility.
- Last visit: Preliminary test on plasma flavonoid contents will be completed within two weeks to make sure you follow the food restriction during the study. **You will be notified to pick up the last payment if you pass the test.**

What are the possible benefits of being in this study?

Research is designed to benefit society by gaining new knowledge. Also, results generated from this project may benefit the general public including participants in this project on the health effects of flavonoid-rich foods. There are no known personal benefits for participating in this study.

What are the possible risks or discomforts involved in being in this study?

There are no major risks associated with the study intervention of soy milk. However, soy consumption may cause some mild stomach and intestinal side effects such as constipation, bloating, and nausea. It may also cause allergic reactions involving a rash, itching, and breathing problems in some people. So, please make sure you have no known allergy to soy milk. If you experience nausea, vomiting or diarrhea, or fevers, you may contact your medical provider and let them know.

Possible risks involved in drawing blood from a vein may mild momentary discomfort at the site of the blood draw, possible mild bruising, redness, and mild swelling around the site, minimal bleeding at the site, mild feeling of lightheadedness when the blood is drawn, and rarely, an infection at the site of the blood draw. There may be uncommon or previously unknown risks. You should report any problems to the researcher. In addition, it is possible that participants may feel a little embarrassed about the collection of urine and stool samples.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue participation.

How will information about you be protected?

All participants will be assigned an ID number, which is used for identification purposes throughout the study. Participant data will be stored on a password-protected university computer, that will be kept locked in the coordinator's office. No data will be placed on any network. The plasma, urine, and fecal samples will be stored in the -80°C freezer and will be labeled with an identifying number. Participants will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if the disclosure is ever required, NC A&T State University will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow the instructions (for example, failed to follow the food restriction during the study), or because the entire study has been stopped.

Will you receive anything for being in this study?
You will be receiving gift card incentives for taking part in this study. The incentives will be split into three parts ($100, $150, and $150) and received depending on the completeness of the study. $100 gift card will be received on the third visit (24 h blood collection after the first arm). Another $150 gift card will be received by the 6th visit (24h blood collection after the second arm). The last payment of $150 gift card will be ready to pick up on the 8th visit (after the preliminary test of plasma flavonoid levels for the food restriction). [1st visit, interview and consent form, food diary form for prewash out. 2nd visit, breakfast, blood, urine; 3rd visit, 24h blood drawing, urine sample, and fecal samples drop off. 4th visit, 48h fecal samples drop off. 5th visit, breakfast, blood, urine, and fecal samples. 6th visit, 24h blood drawing, urine sample, and fecal samples drop off. 7th visit, 48h fecal samples drop off. 8th visit, after the preliminary test.]

Will it cost you anything to be in this study?
It will not cost you anything to be in this study.

What if you are a NCA&T student or employee?
Taking part in this research is not a part of your University duties and refusing will not affect your class standing or grades, or your job. You will not be offered or receive any special consideration if you take part in this research.

What will happen if you are injured by this research?
All research involved a chance that something bad might happen to you. If you are hurt, become sick, or develop a reaction from something that was done as part of this study, the researcher will help you get medical care, but the North Carolina A&T State Univeristy and NIH have not set aside funds to pay you for any such injuries, illness or reactions, or for the related medical care. Adverse events will be reported to the IRB within 72 hours. When medical attention is needed, 911 will be promptly called.

Who is sponsoring this study?
This research is funded by NIH (NIDDK). If you would like more information, please ask the researchers listed on the first page of this form.

What if you have questions about this study?
You have the right to ask and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, or concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

The study results will be published in a scientific journal. You can search for it at any time after it is published.

What if you have any questions about your rights as a research participant?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board (IRB) at 336-334-7995 or by email at rescomp@ncat.edu.

Will I be contacted in the future?

If you choose, you may be contacted for future studies.

Do you consent to be contacted for future studies? Y☐ N☐

Participant's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I also confirm that I do not have an allergy to soy milk intake. I voluntarily agree to participate in this research study.

_____ _____

Signature of Research Participant Date

_____

Printed Name of Research Participant

_____ _____

Signature of Research Team Member Obtaining Consent Date

_____

Printed Name of Research Team Member Obtaining Consent